In- Silico Docking Study of *Terminalia catappa Linn as* a Potent Anti-diabetic Agent

*Olusola Abiola Ladokun, [2]Sarah Olufunsho Oni, [1]Abiola Olasoji Aminu, [1]Okikiola Olabi Durosinlorun, [1]Ayodeji Famuti

[1]Department of Biochemistry, Lead City University, Ibadan, Oyo State, Nigeria

[2] Department of Chemistry, Lead City University, Ibadan, Oyo State, Nigeria

Abstract: Diabetes mellitus is one of the life threatening diseases in Africa, especially in Nigeria which has been shown to have the highest number of people with diabetes with an estimated 3.9 million people. The present study investigate the anti-diabetic active constituent present in *Terminalia catappa Linn* which bind at active site of G-protein coupled bile acid receptor 1 (TGR5), with lowest binding energy using Autodock/Vina 4.2. Molecular screening of phytochemicals constituent found in *Terminalia catappa Linn* reveals that Farnesyl acetone with a docking score of -7.9 kcal/mol in comparison with Cholic acid with a docking score of -7.3kcal/mol which is a standard agonist of G-protein coupled bile acid receptor 1 (TGR5) has high anti-diabetic potency. From the result, it can be concluded that Farnesyl acetone acts as a potent anti-diabetic agent and can be developed into a potent drug for type 2 diabetes.

Keywords: Autodock/Vina, Cholic acid, Diabetes mellitus, Docking, Farnesyl acetone, G-protein coupled bile acid receptor 1 (TGR5), *Terminalia catappa Linn*,

1 Introduction

Diabetes mellitus (DM) is a chronic disease triggered by acquired deficiency or inherited deficiency in the formation of insulin by the pancreas. It is the most widespread metabolic disease worldwide (Abdulazeez, S.S, 2013). DM causes high blood sugar levels over a period of time leading to severe hyperglycemia and complications which include; cardiovascular disease, kidney failure, foot ulcers, diabetic ketoacidosis, and tissue or organ damage.

DM takes an ever-increasing proportion of national and international health care budget as the number of people with diabetes multiply world-wide. In the next 25years, DM is projected to become one of the world's main disablers and killers. Sub-Saharan Africa is estimated to have about 20 million people with diabetes, about 62% are not diagnosed and the number is expected to reach 41.4 million by 2035 or an increase of 109.1%. Nigeria has been shown to have the highest number of people with diabetes with an estimated 3.9 million people of the adult population aged 20-79-year old (World Health Organization; 2013)**.** Diabetes contributes to the development of heart disease, renal disease, pneumonia, bacteraemia and tuberculosis (TB) (Saydah SH, *et al.,* 2002**).** It is shown that people with diabetes are 3 times more likely to develop tuberculosis and approximately 15% of TB globally is estimated to have background diabetes (Jeon CY and Murray MB, 2008)**.**

Management of diabetes without any side effects is still a challenge to the medical system. Having being termed the silent epidemic, diabetes continues to ignite and sustain motivation in finding a cure. This leads to increasing demand for natural products with antidiabetic activity with fewer side effects. However, the potency of the medicinal plants, interaction with other medication, dosage requirements and possible toxicity needs extensive further research. There is also a possibility that medicinal plants used for diabetics may reveal another way of treating the disease that may even be more efficient than current treatments.

TGR5 receptor is the first known G-protein coupled receptor specific for bile acids. TGR5 is expressed in both mouse and human pancreatic cells islets. In pancreatic cells, TGR5 agonists can induce insulin secretion through a cAMP/Ca^{2+} pathway (Kumar DP , *et al.,* 2012*)* . TGR5 receptor agonist enhanced glucose tolerance by lowering glucose and insulin levels (Sato H, *et al.,* 2007). These convergent findings point to bile acid–TGR5 interaction as a key regulator endpoint of basal metabolism regulation (Pols TWH, *et al.,* 2011), (Porez G, *et al.,* 2012*)* . TGR5 is now recognized as a potential target for the treatment of metabolic disorders, such as type 2 diabetes.

Terminalia catappa Linn (Combretaceae) is found throughout the warmer parts of India and called as Indian Almond, Malabar Almond, Tropical Almond. It is a medium sized tree with leaves clustered towards the ends of the branches. The various extracts of leaves and bark of the plant have been reported to be anticancer, antioxidant (Masuda *et al.,* 1999), anti-HIV reverse transcriptase (Tan *et al.,* 1991) and hepatoprotective (Lin *et al.,* 1997), anti-inflammatory (Lin et al., 1999), hepatitis (Chen *et al.,* 2000), and aphrodisiac (Ratnasooriya and Dharmasiri, 2000). They are also reported to contain phytochemicals which are indicative of its potential in treatment of DB (Shimizu *et al.*, 1989).

Bioinformatics tools have become very important to pinpoint the targets for different ligands. Many studies have indicated that computational approaches, such as structural bioinformatics (Chou *et al.,* 2004; Chou *et al.,* 2004) molecular

docking (Wang *et al.,* 2011; Chou et

al., 2003) pharmacophore modelling (Sirois *et al.,* 2004) are best choice. The present study is to investigate the anti-daibetic role of the phytochemicals present in *Terminalia catappa* as a lead target against diabetic in comparison to a standard diabetic drug.

2. Materials and Methods

The docking of *Terminalia catappa Linn* Phytochemical constituent into the binding site of TGR5 receptor was explored using Autodock software, which has been shown to be powerful tools for molecular recognition. To validate the molecular modelling programs, we first evaluated the docking accuracies of Autodock/Vina by docking with known TGR5 receptor agonist, Cholic acid into the binding site.

2.1 Modelling and in silico screening

Ligand (Compound) Preparation for Docking 2-Dimensional structure of the ligand isolated from the plant was drawn using the ChemAxon Software called Marvin Sketch (https://www.chemaxon.com/) while the standard drug Cholic acid acting as an agonist was downloaded from pubchem (https://pubchem.ncbi.nlm.nih.gov/compound/221493#section=Top). To prepare the ligand for molecular docking, the 2D was converted to 3-Dimensional structure with a force field of MMFF94.

2.2 Preparation of protein structure

The 3D structure of G-protein coupled bile acid receptor 1 (TGR5) was downloaded from Protein Data Bank (PDB) (http: //www.pdb.org/pdb/home/home.do), before starting the docking process, all non-protein molecules were removed. TGR5 was modified by adding polar hydrogens and kept rigid in the docking process, where all torsional bonds of the ligands were set free by Ligand module in Autodock/Vina Tools, all docking calculations were performed using Autodock vina 4.2 (Morris *et al.,* 2009). The various ligand in the plant isolated show an excellent basis for using structure-based approaches for the discovery of a new potent drug.

2.3 Molecular docking

AutoDock 4.2 was used to investigate ligand binding to the receptor model using a grid spacing of 2.0 Å and the grid points in X, Y and Z axis were set to 60 × 60 × 60. The grid center was placed in the active site pocket center. The grid boxes included the entire binding site of the enzyme and provided enough space for the ligand translational and rotational walk. At first dock pdb.qt files for protein and ligands were prepared. The search was based on the Lamarckian genetic algorithm and the standard free energy charged was calculated using binding energy. Docking analysis was perform based on binding free energies and root mean square deviation (RMSD) values, and the docking result was ranked in the order of increasing docking energies according to the level of negativity measured in Kcal/mol. The binding energy of each ligand is the main binding energy of all the conformations present with the ligand, the pocket with the lowest binding energy and the higher number of conformations within it was selected as the docked pose of that particular ligand. The ligand were ranked by the lowest-energy representative of each binding mode. The parameters for the docking analysis were set as default values as shown in **(Table 1&2)** .

Table 1: Grid center coordinate

Grid setting	X	Y	Z
	0.97	-22.36	45.51

Table 2: Grid definition parameters

Parameters	Spacing	X-point	Y-point	Z-point
	0.375	60	60	60

2.4 Docking confirmation using Mcule

Mcule speeds up early phase drug discovery by its integrated molecular modelling tools, computational capacity and high-quality compound database (https://mcule.com/dashboard/). Molecular docking using Mcule was done in the Structure-based virtual screen - Workflow builder. The ligand was uploaded in 2D. The receptor which is the target was uploaded in 3D with a binding site center X: 0.97, Y: -22.36, Z: 45.51. The simulation was RUN with a maximum hit of 1000.

2.5 Experimental confirmation and (EC_{50}) Calculation

EC_{50} is the concentration of a drug which induces a response halfway between the base line and maximum after a specified exposure time, it is used as a measure of a drug's potency. Experimental confirmation and EC_{50} calculation was done using Chembl (https://www.ebi.ac.uk/chembl/). The target fasta file was copied from protein data bank (https://www.ncbi.nlm.nih.gov/protein/NP_001308879.1?report=fasta) and uploaded at the Protein target BLAST search in Chembl. The target associated Bioactivities with Target Id of CHEMBL5409 having 53 compound was downloaded. To calculate the EC_{50}, the target associated

Bioactivities was dock against TGR5 receptor with a config.txt parameter **(Table 3)**. After docking the results was harvested by 'egrep'. The Coefficient was determined by plotting a graph shown in ***fig. 3*** of the docked score against the Pchembl value.

Table 3: The config.txt for Chembl moleculer docking using Auto/Vina

Center_x	Center_y	Center_z	Size_x	Size_y	Size_z	Number of modes
0.97	-22.36	45.51	22.50	22.50	22.50	1

3.0 RESULT AND DISCUSSION

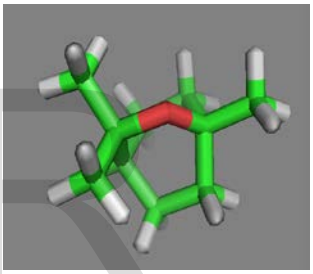
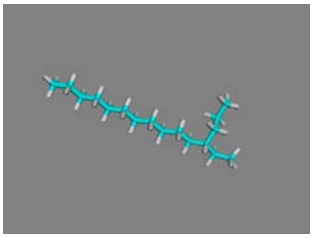
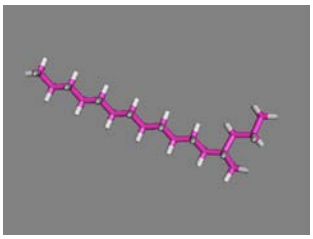
Binding modes to proteins and energies of ligands are commonly predicted by Molecular docking (Bikadi et al., 2009). Docking was done by using Audock/Vina 4.2 which is a suite of automated docking tools used to predict the binding affinity, activity, orientation of the ligands to the target protein molecules TGR5. Experimental analysis was based on the standard free energy of binding, lowest docked energy, and calculated RMSD values. For each docking, the number of hits, the RMSD value of the best hit (with the lowest RMSD) based on shape complementarity are listed in ***Table 5***. Farnesyl acetone was found to bind

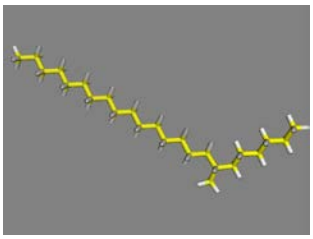
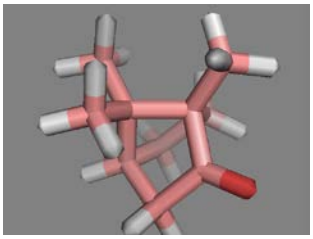
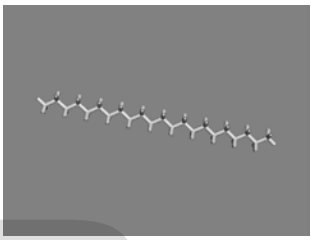
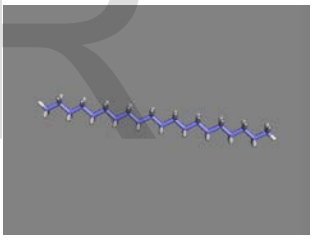
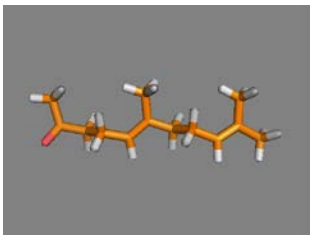
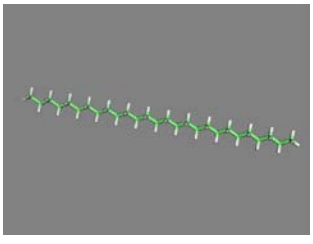
at active site of TGR5 receptor with lowest binding energy band RMSD values to be -7.9Kcal/Mol and 2.0 Å respectively ***fig. 2*** . The mcule ID C-416044812 also gives a docking result of -7.8 Kcal/Mol, which therefore validate the Autodock/Vina docking result. The docked analysis of the standard drug Cholic Acid with TGR5 receptor gives a binding affinity of -7.3Kcal/Mol.

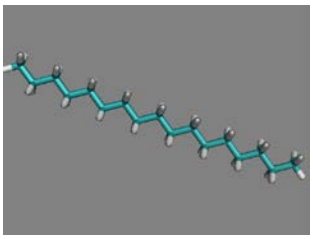
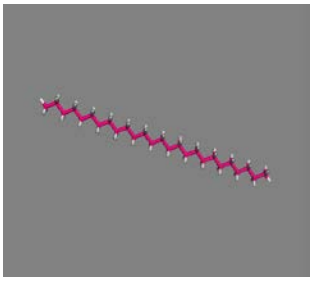
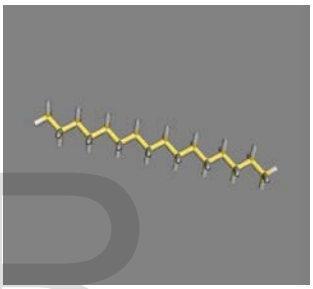
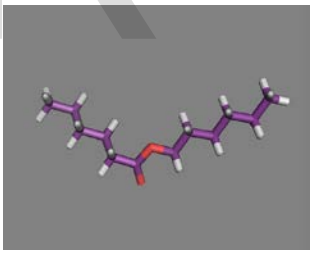
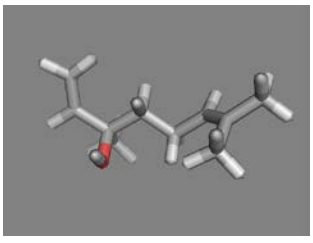
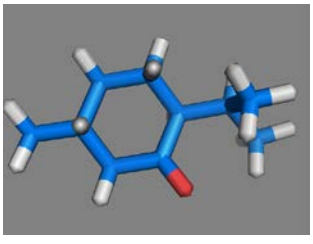
Free energy of binding is calculated as a sum of four energy terms of intermolecular energy (vanderwaal, hydrogen bond, desolation energy and electrostatic energy), total internal energy, torsional free energy and unbound system energy.

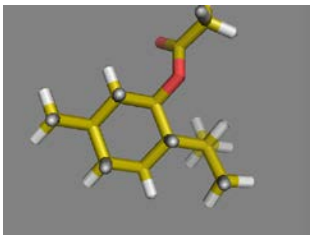
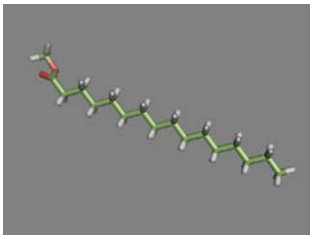
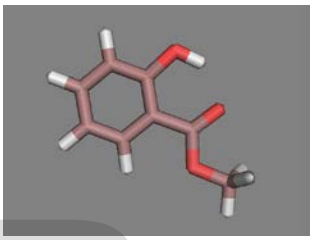
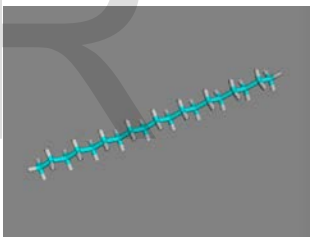
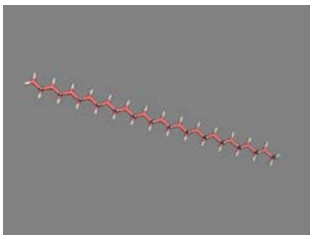
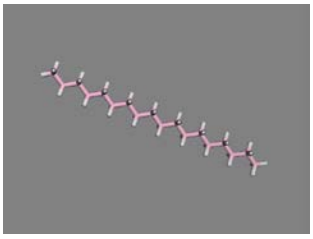
Docking analysis of TGR5 receptor with Farnesyl acetone enabled us to identify specific residues viz. Leu-266, Phe-96, Pro-92, Thr-64, Asn-93, Tyr-89, Tyr-240, Ser-95, Trp-237, Leu-244, Ala-67, Glu-169, Val-88, Thr-70, Leu-173 and Phe-96 within the TGR5 receptor binding pocket to play an important role in ligand binding affinity. The docking of TGR5 receptor and Farnesyl acetone is shown in ***fig. 2***. Our in silico experiments demonstrate that Farnesyl acetone binds TGR5 receptor, and also in itself activate its function and thus may act as a drug.

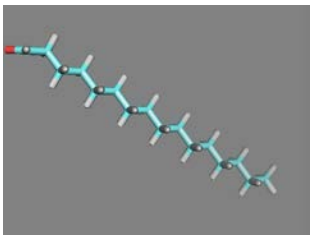
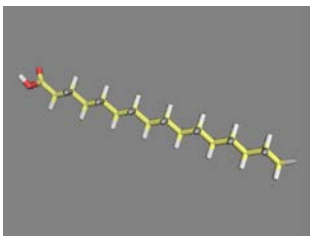
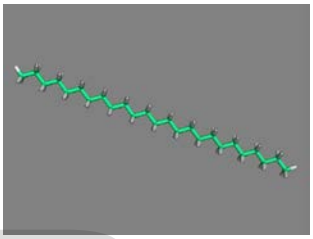
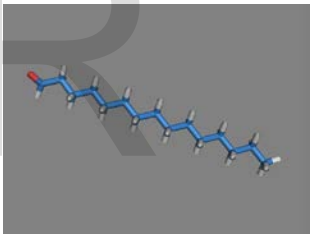
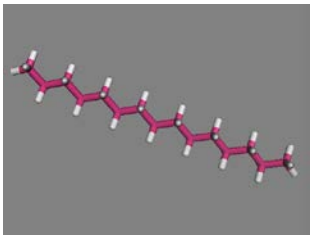
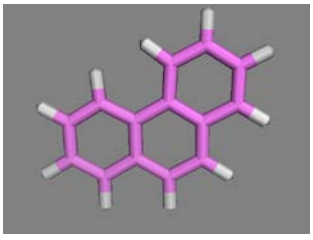
Table 4: Phytochemical constituent of *Terminalia catappa Linn*

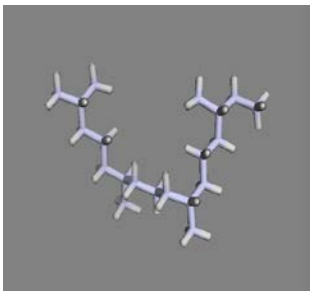
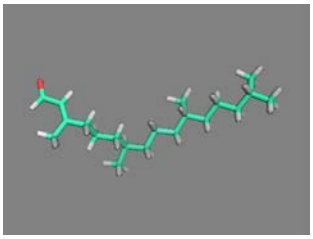
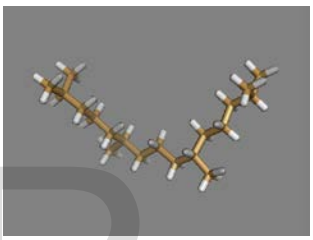
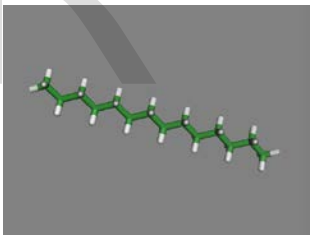
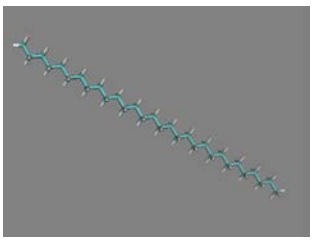
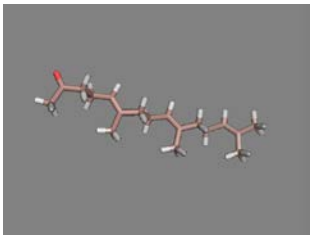
Name	Molecular Weight	Molecular Formula	Structure
1,8-Cineole	154.253 g/mol	$C_{10}H_{18}O$	
4-Ethylpentadecane	240.475 g/mol	$C_{17}H_{36}$	
4-Methylhexadecane	240.475 g/mol	$C_{17}H_{36}$	

7-Methyltricosane	338.664 g/mol	$C_{24}H_{50}$	
Camphor	152.237 g/mol	$C_{10}H_{16}O$	
Docosane	310.61 g/mol	$C_{22}H_{46}$	
Eicosane	282.556 g/mol	$C_{20}H_{42}$	
Geranylacetone	194.318 g/mol	$C_{13}H_{22}O$	
Heptacosane	380.745 g/mol	$C_{27}H_{56}$	

Heptadecane	240.475 g/mol	$C_{17}H_{36}$	
Hexacosane	366.718 g/mol	$C_{26}H_{54}$	
Hexadecane	226.448 g/mol	$C_{16}H_{34}$	
Hexyl hexanoate	200.322 g/mol	$C_{12}H_{24}O_2$	
Linalool	170.252 g/mol	$C_{10}H_{18}O_2$	
Menthone	154.253 g/mol	$C_{10}H_{18}O$	

Methyl_acetate	74.079 g/mol	$C_3H_6O_2$	
Methyl palmitate	270.457 g/mol	$C_{17}H_{34}O_2$	
Methyl salicylate	152.149 g/mol	$C_8H_8O_3$	
Nonadecane	268.529 g/mol	$C_{19}H_{40}$	
Octacosane	394.772 g/mol	$C_{28}H_{58}$	
Octadecane	254.502 g/mol	$C_{18}H_{38}$	

Palmitaldehyde	240.431 g/mol	$C_{16}H_{32}O$	
Palmitic acid	256.43 g/mol	$C_{16}H_{32}O_2$	
Pentacosane	352.691 g/mol	$C_{25}H_{52}$	
Pentadecanal	226.404 g/mol	$C_{15}H_{30}O$	
Pentadecane	212.421 g/mol	$C_{15}H_{32}$	
Phenanthrene	178.234 g/mol	$C_{14}H_{10}$	

Phytane	282.556 g/mol	$C_{20}H_{42}$	
Phytone	268.485 g/mol	$C_{18}H_{36}O$	
Pristane	268.529 g/mol	$C_{19}H_{40}$	
Tetradecane	198.394 g/mol	$C_{14}H_{30}$	
Triacontane	422.826 g/mol	$C_{30}H_{62}$	
Farnesyl acetone	262.437 g/mol	$C_{18}H_{30}O$	

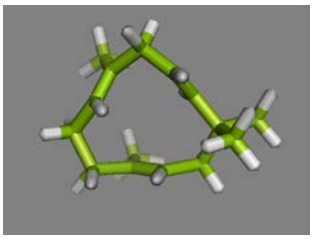
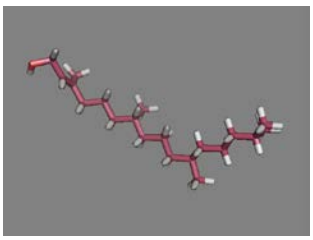
Humulene	204.357 g/mol	$C_{15}H_{24}$	
Phytol	296.539 g/mol	$C_{20}H_{40}O$	

Table 5: Binding affinity of the phytochemical constituent of Terminalia catappa Linn

S/N	Compounds	Binding affinity (Kcal/mol)
1.	1,8-Cineole	-6.3
2.	4-Ethylpentadecane	-6.8
3.	4-Methylhexadecane	-6.3
4.	7-Methyltricosane	-7.0
5.	Camphor	-5.7
6.	Docosane	-6.9

7.	Eicosane	-6.8
8.	Geranylacetone	-7.2
9.	Heptacosane	-7.5
10.	Heptadecane	-6.4
11.	Hexacosane	-6.7
12.	Hexadecane	-6.5
13.	Hexyl hexanoate	-6.3
14.	Linalool	-6.2
15.	Menthone	-6.5
16.	Methyl_acetate	-6.8
17.	Methyl palmitate	-6.4
18.	Methyl salicylate	-5.9
19.	Nonadecane	-6.6
20.	Octacosane	-7.5
21.	Octadecane	-6.7
22.	Palmitaldehyde	-6.6
23.	Palmitic acid	-6.4
24.	Pentacosane	-7.3
25.	Pentadecanal	-6.1
26.	Pentadecane	-6.6
27.	Phenanthrene	-7.6
28.	Phytane	-7.2
29.	Phytone	-7.4
30.	Pristane	-7.3
31.	Tetradecane	-6.2
32.	Triacontane	-7.4
33.	Farnesyl acetone	-7.9
34.	Humulene	-7.0
35.	Phytol	-7.2

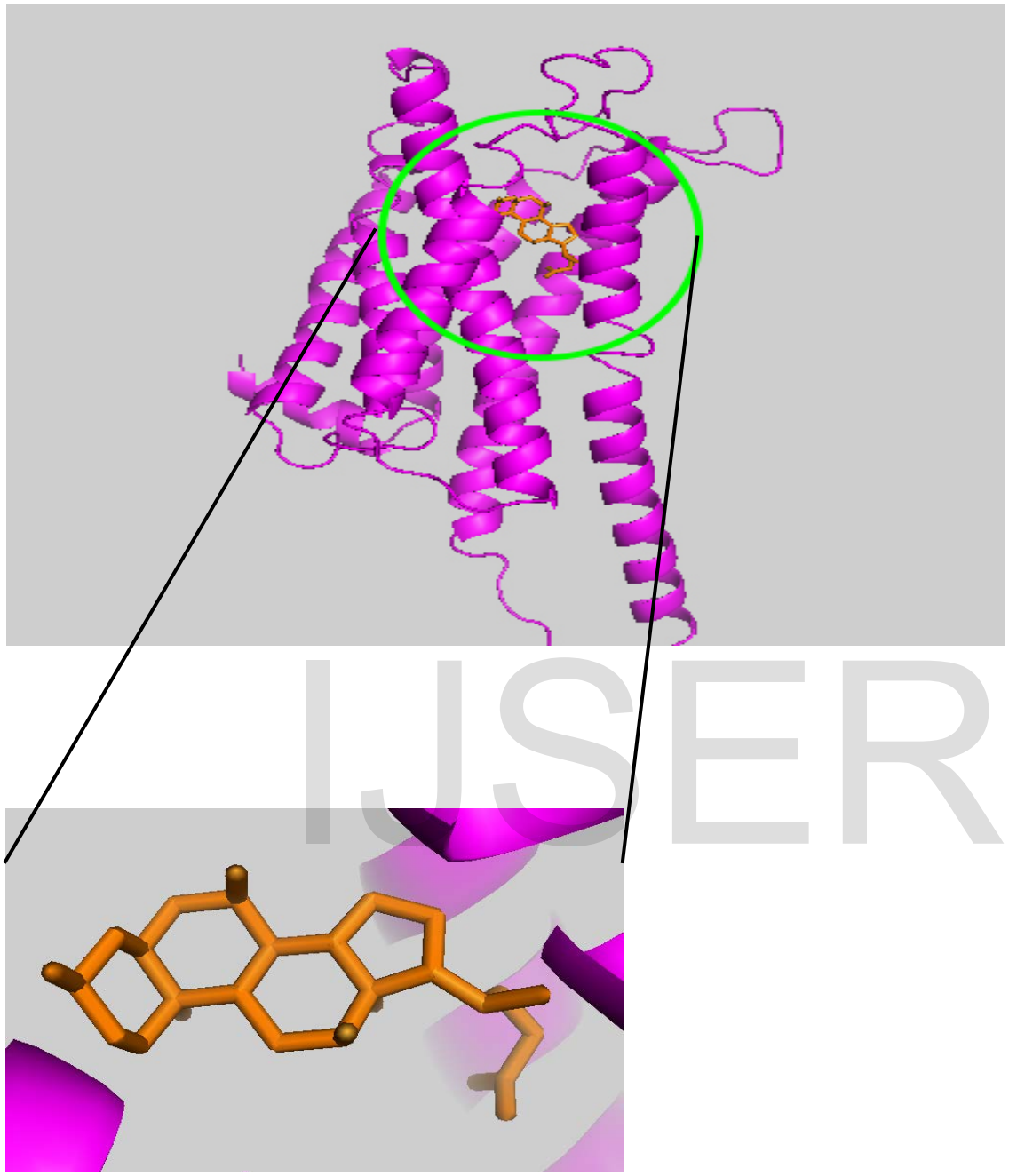
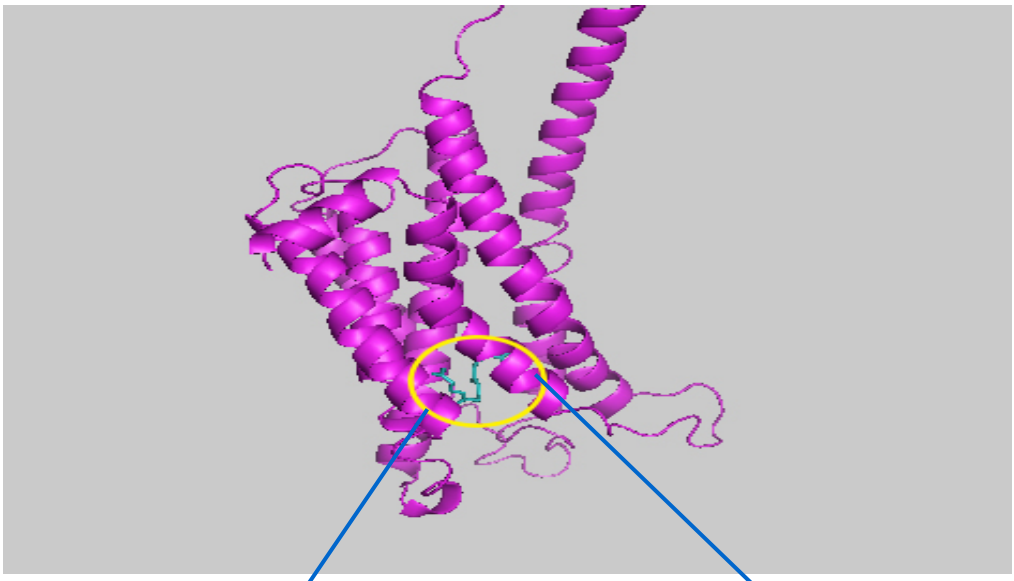
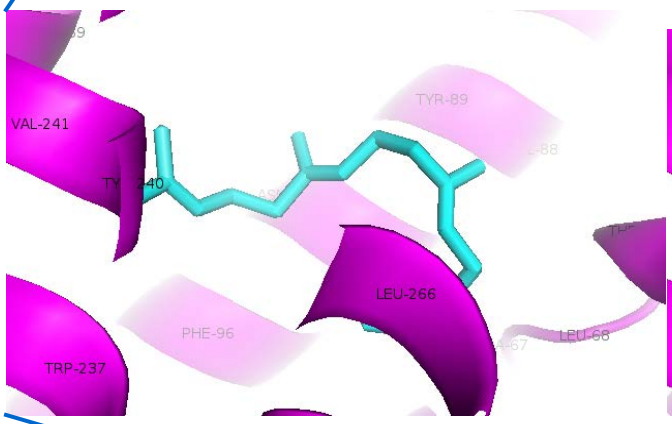


***Figure 1*:** The moleculer binding pose of G-protein coupled bile acid receptor 1 (TGR5) and the standard drug Cholic Acid

a.



b.



c.

***Figure 2*: a.** The moleculer binding pose of G-protein coupled bile acid receptor 1 (TGR5) and Farnesyl acetone **b.** Amono acid residue of G-protein coupled bile acid receptor 1 (TGR5) and Farnesyl acetone **c.** 3D structure of Farnesyl acetone

Figure 3: The coeffient of correlation of EC_{50} G-protein coupled bile acid receptor 1 (TGR5)

4.0 CONCLUSION

Natural compound from plant have been proven to have played an important role in treating and preventing human diseases. In this research work, we compare the binding potency of phytochemical constituent from *Terminalia catappa Linn* with a standard agonist Cholic acid bind to the active site of G-protein coupled bile acid receptor 1 (TGR5). The results of the present study clearly demonstrated the *in silico* molecular docking studies of Farnesyl acetone with a docked score of -7.9 kcal/mol and Cholic acid with a docked score of -7.3 Kcal/mol which therefore validate that Farnesyl acetone is more potent than Cholic acid. Hence, it can be concluded that Farnesyl acetone has high potential as antidiabetic compound. In- vivo and in- vitro approaches is therefore recommended to elucidate the molecular mechanism of this compound to act a potent drug against type 2 diabetes.

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