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ORIGINAL ARTICLE

Molecular Docking Studies on Phyto-Compounds Identified from *Ruellia Tuberosa* Against Protein Tyrosine Phosphatase 1b and Human Aldose Reductase

Aswathi Sreenivasan and Angayarkanni*
Department of Biochemistry, Biotechnology and Bioinformatics, Avinashilingam Institute for Home Science and Higher Education for Women, Coimbatore – 641043
***Corresponding Author**: Angayarkanni
Email: angayarkann_bc@avinuty.ac.in

ABSTRACT
This study aims to determine the effect of compounds present in Ruellia tuberosa L.acanthaceae for multi purpose biological applications. Many compounds were already reported with wide spectrum activities. First part of this work identified that the crude extract of Ruellia tuberosa L.acanthaceae having different biological properties which includes anti-cancer, anti -diabetic, anti- oxidant and anti- bacterial. The second part of the study focuses on In silico approaches to find out the binding properties of compounds. Molecular docking is a crucial technique in structure-based drug design, with the ability to predict binding strength between ligands and protein receptors. This study employsIn silico molecular docking to assess the binding affinities of selected phytochemical compounds with the antidiabetic target enzymes, Protein Tyrosine Phosphatase (PDB ID: 1C88) and Human Aldose Reductase (PDB ID: 4QBX). The research aims to identify potential therapeutic agents for diabetic. In light of the alarming rise in diabetes worldwide, there is an urgent need for innovative treatment options. We compared the docking results of n-Hexadecanoic acid, Phytol, 9,12-Octadecadienoic acid, Octadecanoic acid, and Squalene from plant sources with the standard antidiabetic drug, Glibenclamide, using AutoDock Vina. This widely accepted docking program is known for its reliable results in virtual screening. The findings of this study provide valuable insights into the potential of phytochemical compounds as novel antidiabetic agents, emphasizing their significance in drug design and addressing the escalating diabetes epidemic.
Keywords: *Molecular Docking, Antidiabetic Compounds, Protein Tyrosine Phosphatase, Human Aldose Reductase, Diabetes Mellitus, Ruellia tuberosa*

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INTRODUCTION

Ruellia tuberosa L. acanthaceae also known as Minnie root plant, perennial plant with short lived blue flower.In traditional medicines this plant compound reported with anti-inflammatory, antihypertensive, anti-diabetic, anticancer, antioxidant etc. Different potential compounds which includes phenolic compounds, Oleic acid, methyl esters, steroids, terpenoids, flavonoid etc. In Ayurveda research the plant mentioned as Kiranthinayagam. The grinded leaves applied on skin for dermatology problems. The present investigation examined the binding affinity of a subset of drugs with target receptors involved in hypoglycemic actions. Anti-diabetic crude compounds were screened from the same plant using different *In vitro* methods. Molecular docking is a technique for discovering novel ligands for protein structure that is important in structure-based drug discovery. The capacity to forecast the useful binding strength between the ligand and the receptor complex is a prerequisite for a docking approach [1].*In silico* molecular docking studies are thought to be trustworthy methods for locating target receptors' active regions, examining how compounds attach to proteins, and forecasting the molecules' potential as drugs [2]. Protein tyrosine phosphatase 1B and aldose reductase inhibitors are two potential therapeutic modalities for the treatment of diabetes mellitus and associated consequences. In docking, the scoring

functions alone determine the binding energy of the target proteins and small molecules. To calculate the docking score, the free energy involved in docking is calculated[3].Various online docking software available includes: click docking, Surflex dock, Autodock, Glide, G OLD, LigandScout, FlexX, OEdocking, igemdock, Molegro Virtual Docker, etc.[4].These *In silico* tools plays a vital role in drug designing. Among the various docking software, AutoDock Vina is currently used by many groups worldwide, highly successful docking program, quality of the results and commonly accepted molecular docking for virtual screening . The plant was identified as *Ruellia tuberosa* L.*acanthaceae*. from TNAU, Tamilnadu. The hallmark of diabetes mellitus is persistent hyperglycemia brought on by insufficient insulin secretion, action, or both [5]. Globally, 422 million individuals were estimated to have diabetes in 2016, according to World Health Organization figures. Over the next 20 years, this number might more than double. By 2030 diabetes would be the seventh most popular cause of death in the world (WHO, 2016). Therefore, the present study aims to screen the molecular docking of n-Hexadecanoic acid, Phytol, 9,12-Octadecadienoic acid, Octadecanoic acid and Squalene identified from Ruellia tuberosa with antidiabetic target enzymes as Protein tyrosine phosphatase (PDB ID: 1C88) and Human Aldose Reductase (PDB ID: 4QBX) and compared with standard anti-diabetic drug as Glibenclamide using AutoDock Vina.

MATERIALS AND METHOD

In silico Molecular docking

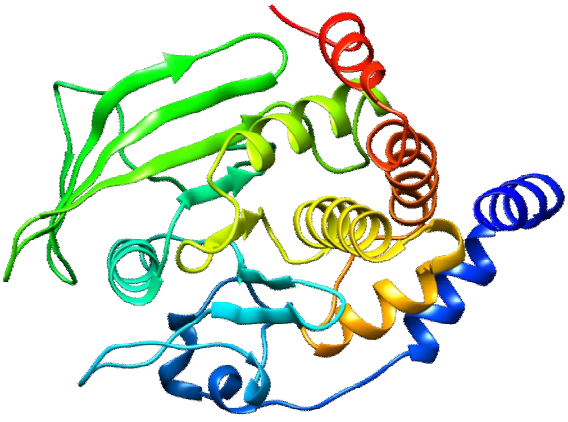
Computational drug discovery technique in the recent day of Pharmaceutical research has successfully molecular modeling with different algorithm-based programing software's been used. Algorithm-based programming software, commonly known as Integrated Development Environments (IDEs), text editors, or code editors, are essential tools for developers and programmers to write, test, and debug their code. The ligand and protein binding scores according to algorithm-based program thereby may use any software for protein and ligand interactions for best results .

Ligand and protein preparation

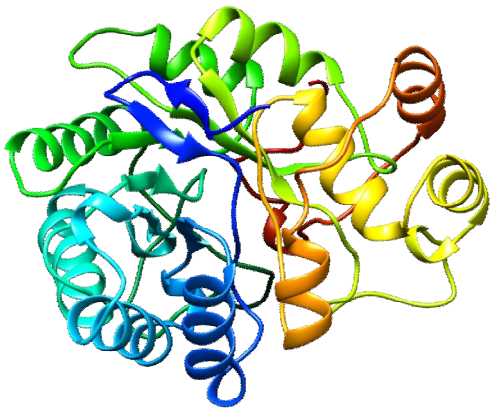
The ligands are n-Hexadecenoic acid, Phytol, 9,12-Octadecadienoic acid, Octadecanoic acid and Squalene and standard antidiabetic drug Glibenclamide were obtained from Pubchem database, ligands were converted in to PDB format using Open bable software and Protein obtained from PDB database. Protein Tyrosine Phosphatase (PDB ID: 1C88) and Human Aldose Reductase (PDB ID: 4QBX) protein preparation was generally to have a remove of all water molecules and any other Ligand molecules prior to docking, using Pymol software prepared protein was saved as PDB formed.

In Silico Docking Studies

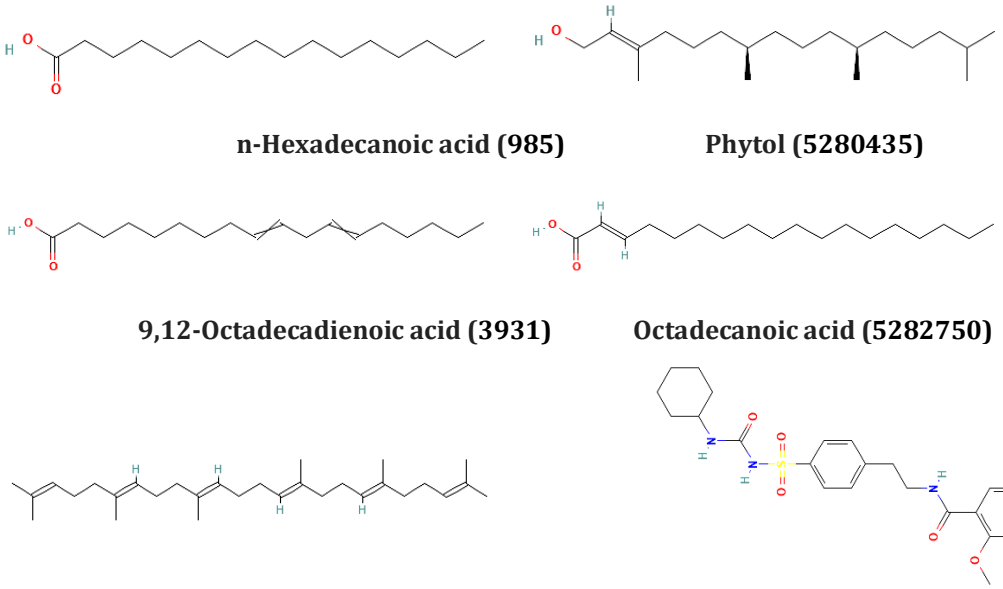
Protein structures were obtained from the Protein Data Bank (PDB), while ligands were sourced from PubChem. Auto Dock Tools with a graphical interface facilitated grid generation, docking score calculation, and evaluation of activator conformers in the protein's active site. Energy minimization was performed using ACD/ChemSketch, and extraneous atoms, including water molecules, were removed from the proteins. AutoDock 4.1, employing a Lamarckian genetic algorithm, estimated interaction energy, allowing torsional flexibility during docking to identify the optimal ligand pose with minimal energy (Figure 1).



PDB ID: 1C88



PDB ID: 4QBX



n-Hexadecanoic acid (985) **Phytol (5280435)**

9,12-Octadecadienoic acid (3931) **Octadecanoic acid (5282750)**

Squalene (638072) **Glibenclamide (3488)**

Figure 1: 2D view of selected ligand and 3D view of target protein

RESULTS

Molecular docking results of phytochemicals with the Protein tyrosine phosphatase (PDB ID: 1C88)

Diabetes mellitus (DM) can be divided into two main categories: insulin-dependent (T1DM) and non-insulin-dependent (T2DM) diabetes. Type 1 DM is associated with the absolute functionality loss of the human pancreatic b-cells to produce insulin, a hormone that regulates glucose. This type of diabetes mainly occurs in childhood. Type 2 DM is related to insulin resistance as well as high glucose and insulin levels at the first stages of the disease. In type 2 diabetic patients, pancreatic b-cells function properly despite the fact that the insulin sensitivity on the target cells is low (Fonseca, 2009). Although more than 90% of adults suffer from T2DM the prevalence of type 2 diabetes has increased among youth. Results are given in Table 1 **,** Figure 2-7.Aldose reductase (ALR2) and protein tyrosine phosphatase 1B (PTP1B) enzymes have been identified as two novel molecular targets involved in different pathways associated with the onset and progression of T2DM and related comorbidities. ALR2 is a key enzyme of the polyol pathway that could induce an excessive accumulation of intracellular reactive oxygen species (ROS) in several tissues resulting in the manifestation of chronic diabetic complications [6]. As far as PTP1B concerns, this enzyme plays a key role in the negative regulation of insulin and the development of insulin-resistance [7]. So far, several reviews have reported a variety of promising therapeutic agents against ALR2 and PTP1B enzymes in the field of diabetes mellitus [8]. Docking engines calculate the Gibbs free energy of binding (ΔG) between a ligand and a receptor, which is fundamental to the understanding of complex systems in biochemistry and molecular biology. The calculation of ΔG is based on estimates of the total energy of intermolecular forces of attraction including van der Waals interactions, hydrogen bonding, and electrostatic interactions. Ligands are ranked by the calculated binding energy value (ΔG); lower binding energy values correspond to more favorable ligand binding, where higher binding energy values are less favorable ligand binding [9].

The higher negative docking score represented a high binding affinity between the receptor and ligand molecules, showing the higher efficiency of bioactive compounds. The docked ligand molecules were selected based on docking energy and good interaction with the active site residues and the results are shown in Table 1. These interactions are represented as two types of arrows from protein to ligand; the first is a dotted green line (H-bond interaction) and the other is a dotted pink line (Alkyl-Hydrophobic interaction). The figure 2 to 7 represent the docking of n-Hexadecanoic acid (-3.70 kcal/mol), Phytol (-4.40 kcal/mol), 9,12-Octadecadienoic acid (-4.90 kcal/mol), Octadecanoic acid (-4.10 kcal/mol), Squalene (-4.40 kcal/mol) and standard antidiabetic drug Glibenclamide (-7.80 kcal/mol). The docking studies confirmed the antidiabetic activity of n-Hexadecanoic acid, Phytol, 9,12-Octadecadienoic acid, Octadecanoic acid and Squalene and thereby inhibition of target protein as Protein tyrosine phosphatase (PDB ID: 1C88) through the binding interactions. The target amino acid residues of proteins interact with

respective ligands represent in table 1. The highest binding energy was greatest activity. Among the various compounds, 9,12-Octadecadienoic acid is highest binding affinity followed by Phytol, Squalene, Octadecanoic acid and n-Hexadecanoic acid. Glibenclamide has the lowest docking score when compared with that of the phytocompounds, Glibenclamide is used as a medication for Type 2 Diabetes. When phytochemical compounds is compared to Glibenclamide, it is observed that its docking capacity of phytocompounds present in *Ruellia tuberosa* is near to that of Glibenclamide. The electrostatic energy of the binding interface is important for protein-ligand complex formation. The binding interactions of all compounds have shown hydrogen bonding and hydrophobic interactions with the target protein. The amino acid residues causing Vanderwaals interactions, hydrogen bond interactions, alkyl and Pi-alkyl interactions as well as the Pi-sigma bonds, which all play a vital role in the protein-ligand stability observed during docking. Hydrogen bonding is one of the most important interactions for biological macromolecular interactions, crucial for conferring stability to protein molecules and selected protein-ligand interactions [10]. The non-bond interactions indicated the alkyl/ Pi-alkyl interaction type which was categorized as a hydrophobic interaction. Hydrophobic interactions play a role in determining the stability of ligands against receptors. Hydrophobic interactions are interactions that avoid the liquid environment and tend to cluster together in the globular structure of proteins .Residues involved in hydrophobic interactions are residues of nonpolar amino acids. Nonpolar (hydrophobic) amino acid residues tend to form clusters in the interior of proteins.

Table 1: Molecular docking results of phytochemicals with the Protein tyrosine phosphatase (PDB ID: 1C88)

Ligand (CID)	Molecular formula	M. weight (g/mol)	H-bond donors / acceptors	Binding Affinity (kcal/mol)	Ligand binding site of target (Protein ID: 1C88) Amino acids
n-Hexadecanoic acid (985)	$C_{16}H_{32}O_2$	256.42	1/2	-3.70	Glu 170, Arg 105, Phe 174, Leu 172, Ser 201, Val 155, Lys 197, Asp 148, Gln 157, Arg 156, Glu 147, Ser 146.
Phytol (5280435)	$C_{20}H_{40}O$	296.50	1/1	-4.40	Ser 205, Gly 202, Leu 204, Arg 79, Arg 199, Glu 200, Phe 196, Phe 280, Asp 236.
9,12-Octadecadienoic acid (3931)	$C_{18}H_{32}O_2$	280.40	1/2	**-4.90**	Lys 248, Asp 245, Val 244, Arg 238, Leu 234, Val 249, Ala 77, Glu 76, Asp 252, Met 74, Glu 75, Lys 255, Phe 256.
Octadecanoic acid (5282750)	$C_{18}H_{34}O_2$	282.50	1/2	-4.10	Glu 101, Glu 97, His 94, Gln 61, Asp 137, Asp 63, Thr 138, Thr 164, Asn 139, His 60, Trp 100, Leu 140, Asn 162.
Squalene (638072)	$C_{30}H_{50}$	410.70	0/0	-4.40	His 54, Phe 30, Lys 255, Phe 256, Lys 73, Leu 71, Ser 55.
*Glibenclamide (3488)	$C_{23}H_{28}ClN_3O_5S$	494.00	3/5	-7.80	His 94, Asn 90, Asp 137, Thr 138, His 60, Glu 97, Trp 100, Leu 140, Glu 101, Asn 162, Asn 139, Thr 164, Glu 62, Asp 63, Gln 61, Asn 64, Arg 43.

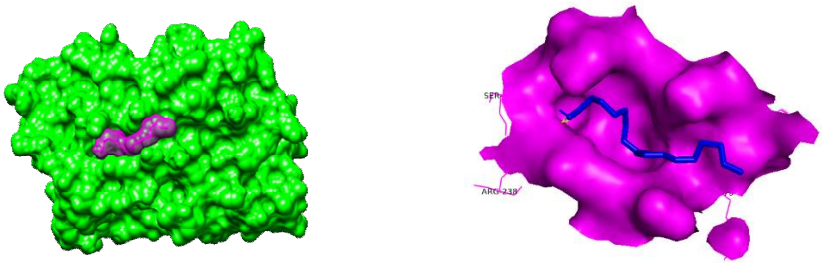
* Standard antidiabetic drug Glibenclamide

Docked complex **Ligand binding site**

Figure 2: Molecular docking of n-Hexadecanoic acid and Protein tyrosine phosphatase (PDB ID: 1C88)

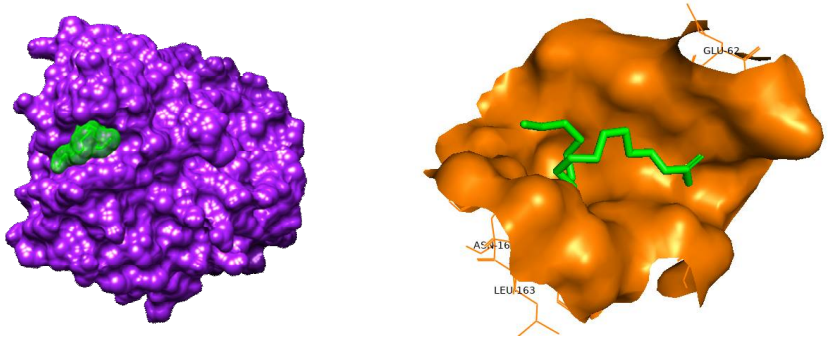
Docked complex **Ligand binding site**

Figure 3: Molecular docking of Phytol and Protein tyrosine phosphatase (PDB ID: 1C88)



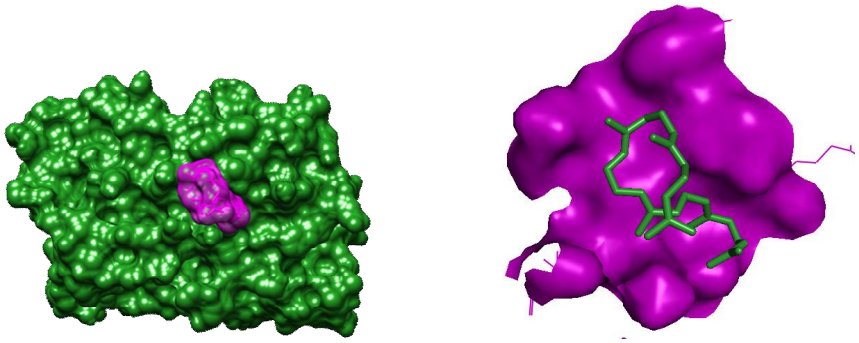
Docked complex **Ligand binding site**

Figure 4: Molecular docking of 9,12-Octadecadienoic acid and Protein tyrosine phosphatase (PDB ID: 1C88)



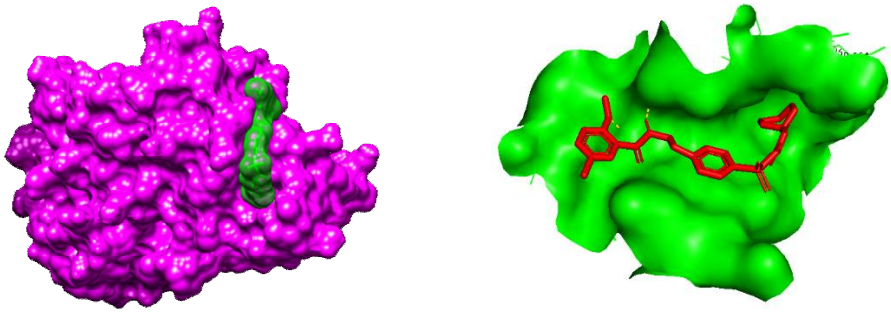
Docked complex **Ligand binding site**

Figure 5: Molecular docking of Octadecanoic acid and Protein tyrosine phosphatase (PDB ID: 1C88)



Docked complex **Ligand binding site**

Figure 6: Molecular docking of Squalene and Protein tyrosine phosphatase (PDB ID: 1C88)



Docked complex **Ligand binding site**

Figure 7: Molecular docking of Glibenclamide and Protein tyrosine phosphatase (PDB ID: 1C88)

Molecular docking results of phytochemicals with the Human Aldose Reductase (PDB ID: 4QBX)

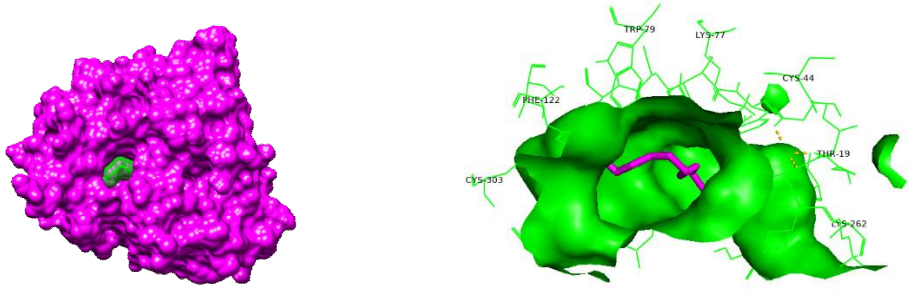
The higher negative docking score represented a high binding affinity between the receptor and ligand molecules, showing the higher efficiency of bioactive compounds. The docked ligand molecules were selected based on docking energy and good interaction with the active site residues and the results are shown in Table 2. The figure 8 to 13 represent the docking of n-Hexadecanoic acid (-7.20 kcal/mol), Phytol (-7.80 kcal/mol), 9,12-Octadecadienoic acid (-6.80 kcal/mol), Octadecanoic acid (-7.80 kcal/mol), Squalene (-10.00 kcal/mol) and standard antidiabetic drug Glibenclamide (-11.20 kcal/mol). The binding interactions of all compounds have shown hydrogen bonding and hydrophobic interactions with the target protein. The docking studies confirmed the antidiabetic activity of n-Hexadecanoic acid, Phytol, 9,12-Octadecadienoic acid, Octadecanoic acid and Squalene and thereby inhibition of target protein as Human Aldose Reductase (PDB ID: 4QBX) through the binding interactions. The highest binding energy was greatest activity. Results are given Table 2 and Figure 8-12.Among the various compounds, Squalene has binding energy followed by Phytol, Octadecanoic acid, n-Hexadecenoic acid and 9,12-Octadecadienoic acid.

Table 2: Molecular docking results of phytochemicals with the Human Aldose Reductase (PDB ID: 4QBX)

Ligand (CID)	Molecular formula	M. weight (g/mol)	H-bond donors / acceptors	Binding Affinity (kcal/mol)	Ligand binding site of target (Protein ID: 4QBX) Amino acids
n-Hexadecanoic acid (985)	$C_{16}H_{32}O_2$	256.42	1/2	-7.20	Asp 43, Ile 260, Lys 77, Gly 18, Thr 19, Lys 262, Ser 210, Cys 298, Tyr 209, His 110, Tyr 48, Trp 20, Val 47, Trp 111, Leu 300, Phe 122.
Phytol (5280435)	$C_{20}H_{40}O$	296.50	1/1	-7.80	Phe 115, Phe 122, Leu 300, Trp 111, Trp 79, Ala 299, Val 47, Trp 20, Tyr 48, Lys 77, Ser 159, Asn 160, Gln 183, Lys 21, Thr 19, Lys 262, Gly 18, Ser 210, Tyr 209, His 110, Trp 219, Cys 298.
9,12-Octadecadienoic acid (3931)	$C_{18}H_{32}O_2$	280.40	1/2	-6.80	Tyr 48, His 110, Phe 121, Leu 300, Phe 115, Thr 113, Phe 122, Trp 111, Trp 219, Cys 298, Trp 79, Val 47, Trp 20.
Octadecanoic acid (5282750)	$C_{18}H_{34}O_2$	282.50	1/2	-7.80	Phe 115, Thr 113, Cys 303, Leu 300, Phe 122, Trp 111, Trp 79, His 110, Trp 219, Tyr 209, Cys 298, Trp 20, Asp 43, Gln 183, Ser 210, Ser 214, Lys 262, Lys 21, Ile 260, Tyr 48.
Squalene (638072)	$C_{30}H_{50}$	410.70	0/0	**-10.00**	Pro 261, Ser 210, Tyr 48, Tyr 209, Val 47, Ala 299, Pro 310, Phe 311, Cys 303, Thr 113, Phe 115, Tyr 309, Leu 300, Trp 219, Trp 111, Trp 79, Asn 160, His 110, Phe 122, Cys 298, Trp 20, Asp 43, Thr 19, Ile 260, Lys 21, Gly 18, Asp 216, Pro 215, Lys

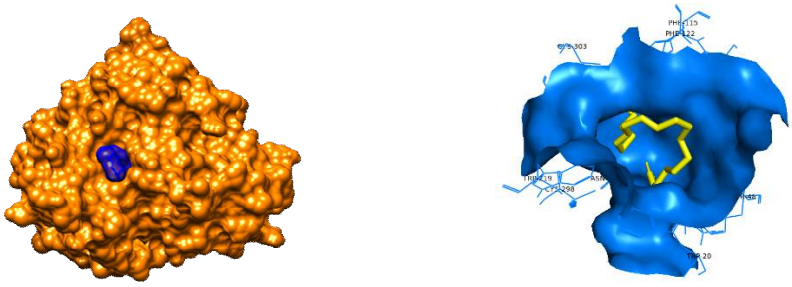
					262, Ser 214, Leu 212, Pro 211.
*Glibenclamide (3488)	$C_{23}H_{28}ClN_3O_5S$	494.00	3/5	-11.20	Pro 215, Leu 212, Ser 214, Lys 262, Asp 216, Ile 260, Lys 21, Gly 18, Tyr 209, Asp 43, Trp 79, Phe 122, Gln 183, Asn 160, Cys 298, Leu 300, Trp 219, Pro 218, His 110, Val 47, Trp 20, Tyr 48, Thr 19, Lys 77, Ser 210, Pro 211, Pro 261.

* Standard antidiabetic drug Glibenclamide



Docked complex **Ligand binding site**

Figure 8: Molecular docking of n-Hexadecanoic acid and Human Aldose Reductase (PDB ID: 4QBX)

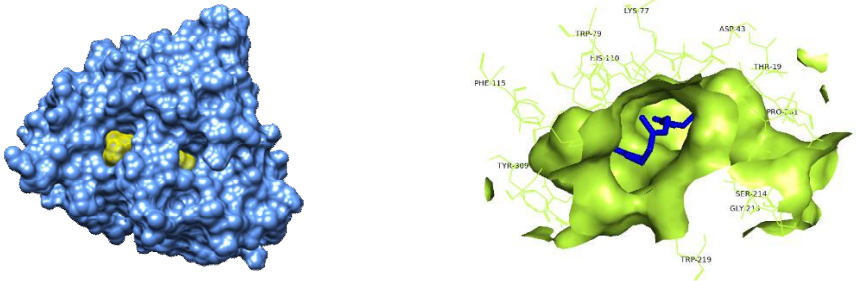


Docked complex **Ligand binding site**

Figure 9: Molecular docking of 9,12-Octadecadienoic acid and Human Aldose Reductase (PDB ID: 4QBX)

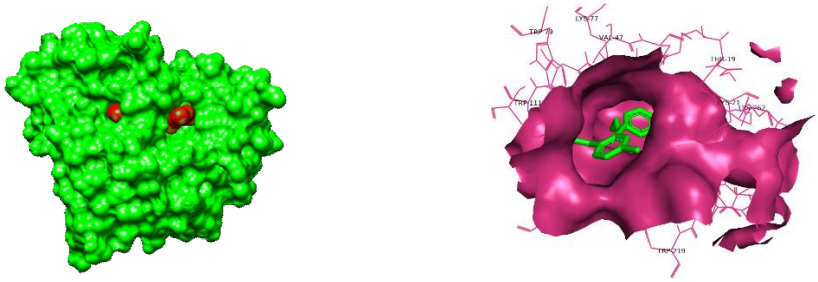
Docked complex **Ligand binding site**

Figure 10: Molecular docking of Octadecanoic acid and Human Aldose Reductase (PDB ID: 4QBX)



Docked complex **Ligand binding site**

Figure 11: Molecular docking of Squalene and Human Aldose Reductase (PDB ID: 4QBX)



Docked complex **Ligand binding site**

Figure 12: Molecular docking of Glibenclamide and Human Aldose Reductase (PDB ID: 4QBX)

DISCUSSION

This study showed the bioactive components of therapeutic potentials in *Ruellia tuberosa* while creating a platform for screening, isolating and identifying many bioactive components which may be useful in the treatment of the various ailments, disorders and dises in the nearest future High-throughput screening (HTS) of compound libraries is used to discover novel leads for drug development. When a structure is available for the target, computer-based screening using molecular docking may also be considered. The two techniques have rarely been used together on the same target. The opportunity to do so presented itself in a project to discover novel inhibitors for the enzyme protein tyrosine phosphatase-1B (PTP1B), a tyrosine phosphatase that has been implicated as a key target for type II diabetes. A corporate library of approximately 400 000 compounds was screened using high-throughput experimental techniques for compounds that inhibited PTP1B. The present study has found that the selected bioactive compounds, including n-hexadecanoic acid, Phytol, 9,12-Octadecadienoic acid, Octadecanoic acid, and Squalene, demonstrate efficient binding to receptors. This indicates that molecular docking can successfully aid in the discovery of inhibitors for protein tyrosine phosphatase (PTP1B) and aldose reductase. Glibenclamide is used as a medication for Type 2 Diabetes. When phytochemical compounds are compared to Glibenclamide, it is observed that its docking capacity is similar/near to that of Glibenclamide. Therefore, it indicates that there are phytocompounds in Ruellia tuberosa with drug-targeting activity. This suggests that *Ruellia tuberosa* could be used for diabetic treatment. The findings suggest that using the same plant crude extract for antidiabetic tests on specific cell lines could potentially lead to the development of a phytoremedy. This phytoremedy might offer a natural solution for managing diabetic conditions. In other words, the research indicates that the plant extract, when tested on relevant cell lines, has shown promise in potentially becoming a natural remedy that could be used to help control diabetes. This highlights the potential for using natural plant extracts as a means of managing diabetic conditions, which could be an important and promising avenue for further research and development.

CONCLUSION

The results of this investigation indicate that n-hexadecanoic acid, Phytol, 9,12-Octadecadienoic acid, Octadecanoic acid, and Squalene are among the bioactive substances that exhibit effective receptor binding. This suggests that the development of inhibitors for aldose reductase and protein tyrosine phosphatase (PTP1B) can be effectively aided by molecular docking. Glibenclamide is a type 2 diabetes treatment drug. Phytochemical substances exhibit a docking capacity that is comparable to or almost identical to Glibenclamide when compared. Consequently, it suggests that Ruellia tuberosa contains phytocompounds with drug-targeting properties. This implies that treating diabetes may benefit from the usage of *Ruellia tuberosa.*

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