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Evaluation Antihyperlipidemic Activity of Garcinia Indica Leaves onIsolated Compounds by Preliminary Molecular Docking and In-SilicoADME Properties

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ABSTRACT: The present study was carried out "Evaluation Antihyperlipidemic Activity of Garcinia Indica Leaveson Isolated compounds by Preliminary Molecular Docking and In-SilicoADME Properties".Molecular docking is a key tool in structural molecular biology and computer-assisted drug design. The goal of ligand-protein docking is to predict the predominant binding mode(s) of a ligand with a protein of known three-dimensional structure. Successful docking methods search high-dimensional spaces effectively and use a scoring function that correctly ranks candidate dockings. Docking can be used to perform virtual screening on large libraries of compounds, rank the results, and propose structural hypotheses of how the ligands inhibit the target, which is invaluable in lead optimization. The setting up of the input structures for the docking is just as important as the docking itself, and analysing the results of stochastic search methods can sometimes be unclear. This chapter discusses the background and theory of molecular docking software and covers the usage of some of the most-cited docking software.

KEYWORDS: Garcinia Indica,Hyperlipidemia, Molecular Docking, ADME, Drug Likeness.

I. INTRODUCTION:

Hyperlipidemia is a metabolic disorder, specifically characterized by alterations in serum lipid andlipoprotein profile due to increased concentration of Total Cholesterol (TC), Low-Density LipoproteinCholesterol(LDL-C),VeryLow-DensityLipoproteinCholesterol(VLDL-C),andTriglycerideswitha concomitant decrease in the concentration of High-Density Lipoprotein Cholesterol (HDL-C) in theserum.[1][2]

Hyperlipidemia has been considered as one of the most important risk factors contributing to theincreased prevalence of coronary heart disease, atherosclerosis and ischemic heart disease. Accordingto WHO reports, high blood cholesterol contributes to approximately 56% of cardiovascular diseasesworldwide and causes 4.4 million death every year. By the year 2030, it is estimated that almost 23.6million people will die from cardiovascular diseases mainly from heartdisease and stroke. A 50%reductioninheartdiseaseswasobservedwhenthelevelsofserumcholesterolwerereducedby10%inmenaged40,similarly,a 20%reductioninheartdiseasescanbeobservedinmenages70bythesameserumcholesterolreduction.Epidemiologicalstudieshaveshownthatthereisa directrelationshipbetween serumcholesterolandcoronary artery disease (CAD).[3][4][5]

Hyperlipidemia is the underlying cause of cardiovascular diseases, CHDs and atherosclerosis. Antihyperlipidemic agents have significant potential to retard the process of atherosclerosis therefore they have been increasingly used as prophylactic in the above disorders associated with hyperlipidemia. Currently available drugs to treat people with detrimental lipid levels include statins, fibric acid derivatives, bile acid-binding resins, and cholesterol absorption inhibitors.[6]

Isolation of active constituents and synthesizing them to target the receptors is a tedious process. The alternative is to make it possible using in-silico studies. There are various software's like Dock, Auto dock, Argus lab, Glide, Gold, Maestro, etc. and various supporting softwares like Chemdraw, Chemsketch, Python, Molgrow, etc. available which aid the drug discovery process and

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make it less tedious.[7]Docking is a search database of molecular structures and retrieves all molecules that can interact with the molecule of interest. It attempts to find the best matching between two molecules. Docking is important to find inhibitors for specific target proteins and to design new drugs. It is acquiring importance as the number of protein structures increases and the efficiency increases accordingly.

Some of the successful outcomes of docking studies are the discovery of Amprenavir (Agenerase) for HIV protease inhibition by GSK and Vertex, Nelfinavir (Viracept) for HIV by Pfizer and Zanamivir (Relenza) for influenza neuraminidase inhibitor by GSK.[8]

This study also attempts to evaluate in-silico antihyperlipidemic activity, mechanism of action, ADME properties and toxicity profiles for some of the isolated selected compounds of Garcinia Indica Leaves.

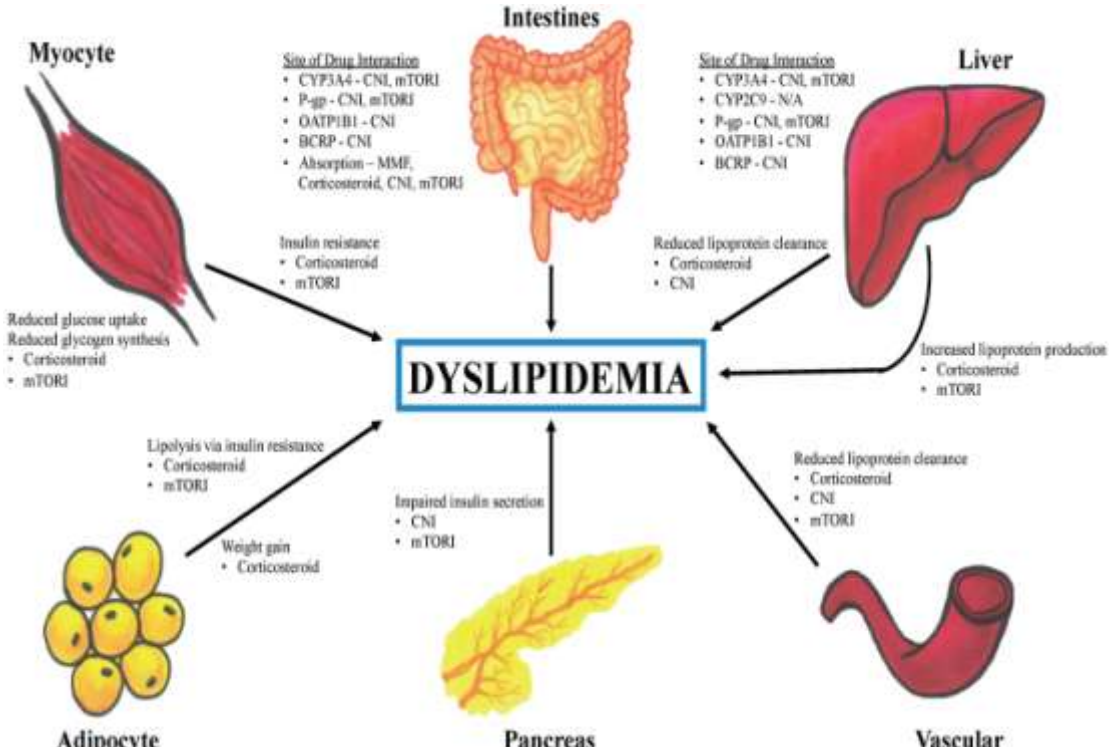

Figure No. 1 Pathophysiology of Dyslipidemia

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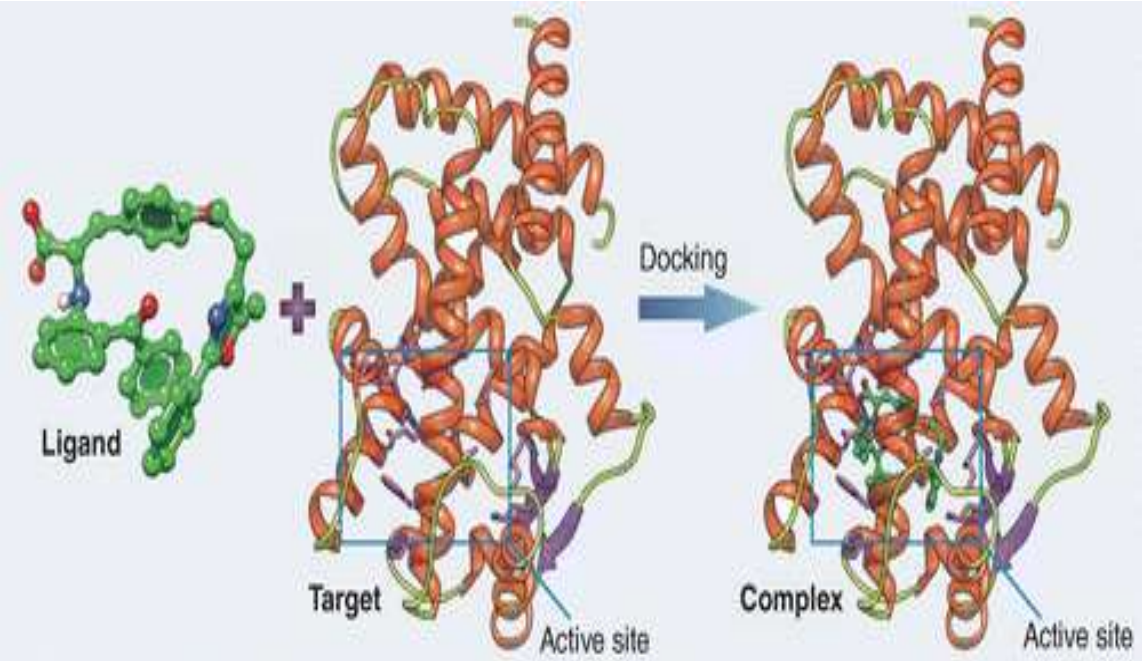

Figure No.2 Docking Process

Types of drug design:[9]

- Ligand-based drug design
- Structure-based drug design

Ligand-based drug design:

Ligand-based drug design is an indirect approach which relies on knowledge of other molecules that bind to the biological target of interest. These other molecules may be used to derive a pharmacophore model that defines the minimum necessary structural characteristics a molecule must possess in order to bind to the target. In other words, a model of the biological target may be built based on the knowledge of what binds to it and this model, in turn, may be used to design new molecular entities that interact with the target.

Structure-based drug design:

Structure-based drug design is a direct approach which relies on knowledge of the three-dimensional structure of the biological target obtained through methods such as x-ray crystallography and NMR spectroscopy. If an experimental structure of a target is not available, it may be possible to create a homology model of the target based on the experimental structure of a related protein. Using the structure of the biological target, candidate drugs that are predicted to bind with high affinity and selectivity to the target may be designed using interactive graphics. This combined with the intuition of a medicinal chemist helps in the suggestion of new drug candidates.

I. EXPERIMENTATION

Requirements:

- **Software Required:**
- Autodock Tools
- Mgl Tools
- Chemdraw/Kingdraw
- Marvin Sketch
- PyMOLWin
- Marvin View
- Discovery Studio
- Autodock Vina 1.1.2

IN SILICO STUDIES

A review of the literature showed that fruits of Garcinia Indica Leaves, have the phytochemicals like alkaloid, phenolic compounds, flavonoid, sapogenin, steroidal alkaloid, glycoside, carbohydrate, steroids, amino acids, etc. The alkaloid, phenolic compounds, flavonoid, glycoalkaloid, sapogenin, steroidal alkaloids were taken up for in silico toxicity, docking and drug-likeness studies.

Isolated compounds are taken for this study include

1) Garcinol
2) Isogarcinol
3)Hydroxy-Citric Acid

A. In silico toxicity prediction

Toxicity screening is done in silico using **OSIRIS** property explorer. It is a web-based

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software available on the Organic Chemistry Portal. Using this prediction tool, mutagenicity, tumorogenicity, skin irritancy, and reproductive effects can be calculated. The prediction properties depend on a precompiled set of structure fragments that gives rise to toxicity alerts if they are found in the structure currently drawn. These fragment lists are created by rigorously shredding all compounds in the database known to be active in a certain toxicity class. During the shredding, any molecule is first cut at every rotatable bond leading to a set of cores fragments.[10] OSIRIS software is used to calculate various drug-relevant properties of chemical structures. The results are color-coded. The green color represents that the compound is non-toxic. Yellow and red color indicates moderate and severe toxicity of the chemical respectively.[11]

B. Docking

- **Preliminary molecular Docking Studies**

The molecular docking studies of all the isolated compounds were done at Bharati Vidyapeeth's college of pharmacy, C.B.D, Belapur, Navi Mumbai.

- **Selection of Binding site on the receptor**

Bioinformatics tools like molecular docking experiments, which involve the study and analysis of ligand-receptor interactions, play important role in identifying molecular targets for different ligands. Novel molecular targets for antihyperlipidemic drugs have been periodically reviewed. There are various molecular targets for antihyperlipidemic activity. Each molecular target has been the individual mechanism of action. Molecular targets for antihyperlipidemic activity are

- Niemann Pick C1 Like 1 protein (NPC1L1) - Reduces the absorption of cholesterol[12]
- ATP citrate lyase (ACL) - Supply Ach-Co-A which is important for cholesterol biosynthesis[13]
- C-reactive protein (CRP) - Damages LDL
- Lanosterol 14α- demethylase(LDM)- Catalyse the cholesterol biosynthesis[14]
- Squalene synthase (SqS) - Key cholesterol precursor [15]
- Farnesoid X-receptor (FXR) - Cholesterol metabolism [16]

The molecular target NPC1L1 was chosen for docking some of the isolated compounds of Garcinia Indica Leaves. NPC1L1 is a gene associated with NPC1 which mutation results in Niemann-Pick disease. It codes for Niemann-Pick C1-like protein 1, found on the gastrointestinal tract epithelial cells as well as in hepatocytes. Specifically, it appears to bind to a critical mediator of cholesterol absorption.[17]

There are various Protein Data Banks for NPC1L1 (3QNT, 3GKH, 3GKI, 3GKJ, 3GCW, 3GCX, 3BPS). The PDB file was selected based on its species, X-ray crystallography or NMR spectroscopy, resolution value, external ligand and presence of co-factors. 3GCX was taken for this study.

During our literature survey, we found that herbal drug shows antihyperlipidemic activity on 3GCX A site of the receptor. Therefore, we decided to perform docking studies on the A binding site of the receptor.

Procedure:

Preliminary molecular docking was performed by using Autodock Vina software on PC with Intel(R)Core (TM)i5-1035G1 CPU @ 1.00GHz 1.19GHz processor and Windows 10 operating system 21H2. The crystal structure of 3GCX and 3QNT was retrieved from the RCSB protein data bank (3GCX PDB ID: PCSK9: EGFA with resolution 2.70Aº) (3QNT PDB ID: NPC1L1 (NTD) with resolution: 2.83Aº)

In this study, Glide (Grid based Ligand Docking with Energetics) program was used for screening the isolated compounds. Glide automatically searches for favorable interactions between ligand molecules and the receptor in different conformations. The docking procedure using Glide includes the following steps,

1. Protein preparation
2. Receptor grid generation
3. Ligand preparation
4. Ligand docking
5. Visualizing docking poses

1. Protein preparation

Protein data bank (PDB) file, which is the crystallized structure of the receptor/ protein is imported from Protein data bank with the following **PDB Id: 3GCX**, resolution **2.7Å**, preprocessed involving the addition of hydrogen, assigning bond order, finding overlaps, creating a zero-order bond to metals, creating disulfide bonds, filling missing side chains and loops using the prime option. The water molecules, co-factors and unwanted chains were deleted. The energy minimization was done to make it ready for grid generation. The PDB file was selected based on its species, X-ray crystallography

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or NMR spectroscopy, resolution value, external ligand and presence of co-factors.[18]

2. Receptor grid generation

After the preparation of protein, the grid has to be generated which is the critical process. It includes defining the active site in the protein (receptor). The prepared protein file was loaded into the workspace. The active site residues were found and picked and the length for docking the ligand to the protein is given as 10Å. The grid was generated by pressing "start" in the grid generation tab. The grid output file obtained as a zip file format was utilized for further docking process.

3. Ligand preparation

The ligand preparation process consists of a series that include conversions, applying corrections to the structures drawn, generating variations on the structure, eliminating unwanted structures and optimizing the structures. Variations on the structure can be made by the addition of hydrogen atoms, removal of unwanted molecules, neutralizing changed groups. The structure can be optimized by generating ionization states, generating tautomers. filtering their structure based on Lipinski's rule of five.

4. Ligand docking

After the generation of the grid, the prepared ligands were docked to see the interaction with the active site of the protein. There were hydrophobic, hydrophilic and Van der Waal's interactions. The strength of the interaction was different ligand molecules. During the docking procedure, the conformation of the ligand was retained and extra precision (XP) mode was selected. In this procedure, the following constraints like the active site and rotatable groups have been checked.[19]

5. Visualization of the docking poses

Once the molecules were docked, then they were visualized for interactions, score and some other parameters like log P value and ionization value. There were interactions like hydrogen bonding, hydrophobic interaction, Van der Waal's interaction between the receptor and the ligand. Based on the interaction and score obtained, the molecules were categorized into hit and flop.

D. In silico screening of drug-likeness

For a drug to be pharmacologically active and exert action it should posses pharmacokinetic properties like absorption, distribution, metabolism and excretion. Many drug failures occur due to unfavorable ADME properties in the field of drug research and development. This has to be ruled out earlier in the process of drug discovery. Some computational methods (in silico tools) have been evolved to investigate the most suitable drug molecules before synthesis.

Lipinski's rule of five also known as **Pfizer's rule of five** is a rule to evaluate drug-likeness. It is used to predict whether a molecule is likely to be orally bio-available or to evaluate drug-likeness.

The designed and docked molecules are screened in silico using **Molinspiration Cheminformatics Software** to evaluate drug-likeness. This tool is quick and easy to use. It can be accessed online for calculation of important molecular properties such as log P, polar surface area, number of hydrogen bond donors and acceptors as well as prediction of bioactivity score for the most important drug targets like GPCR ligands, kinase inhibitors, ion channel modulators, nuclear receptors.[19]

II. RESULTS AND DISCUSSION:

In silico studies

A. Toxicity studies

Toxicity is one of the major criteria to be considered for a molecule to shine as a successful clinical candidate in pharmaceutical research. So, the toxicity studies of some of the isolated molecules of Garcinia Indica Leaves were performed. Toxicity was predicted by the **OSIRIS** Property Explorer, Pass prediction, the online software. Properties with high risks like mutagenicity, reproductive effect, tumorigenicity, and skin irritancy are shown in red color whereas a green and orange color indicates non-toxic behavior of the drug. Toxicity parameters are tabulated in **Table 1**

Sr. No.	Molecules	Mutagenicity	Tumorigenicity	Irritant	Reproductive Effect
1	Garcinol	-	-	-	-
2	Isogarcinol	-	-	-	-

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3	**Hydroxy-citric Acid**	**-**	**-**	**-**	**-**

A)

B)

C)

Figure No. 3 Non-Toxicity of Isolated Compounds A) Garcinol B) Isogarcinol C) Hydroxy-citric Acid

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Sr. No.	Compounds Name	Docking Score (kcal/mol)
1	Garcinol	-6.6
2	Isogarcinol	-7.1
3	Hydroxy-citric Acid	-5.1

B. Docking

Preliminary Docking Study

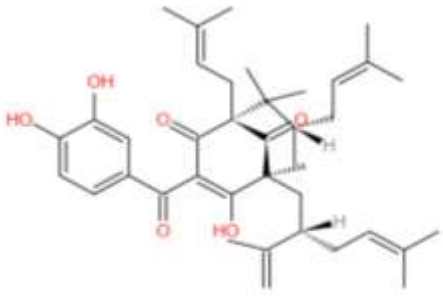
Molecular docking has become a standard tool in computational chemistry for predicting the binding orientation of small molecule drug candidates with their protein targets in order to predict the affinity and activity of the small molecule. Thus, molecular docking plays an important role in the rational design of drugs. Preliminary molecular docking has helped researchers to virtually screen a library of ligands (or compounds) against a target protein and predict the binding conformations and affinities of the ligands to thetarget.

The preliminary docking simulation studies of the various isolated compounds were performed and results were obtained. Among various designed derivatives compounds with good docking scores, Dock poses, and 3D binding.

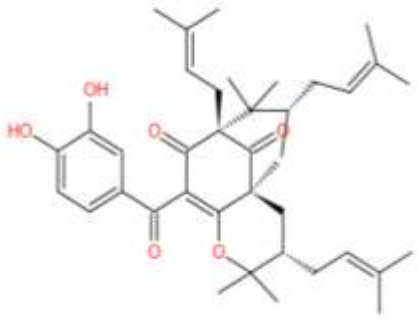
It is considered that the lesser the docking score value greater is the binding of the ligand with the protein. From the docking score values, it is observed that Garcinol, Isogarcinol and Hydroxy-citric Acid showed a docking score value ranging between -7.5 to -5.0 indicating a good score.

Structure of Isolated Compounds

A) Garcinol
B) Isogarcinol
C) Hydroxy-citric Acid



A)



B)

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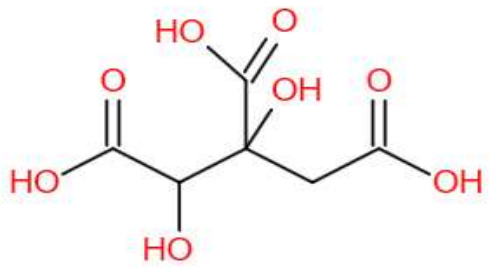
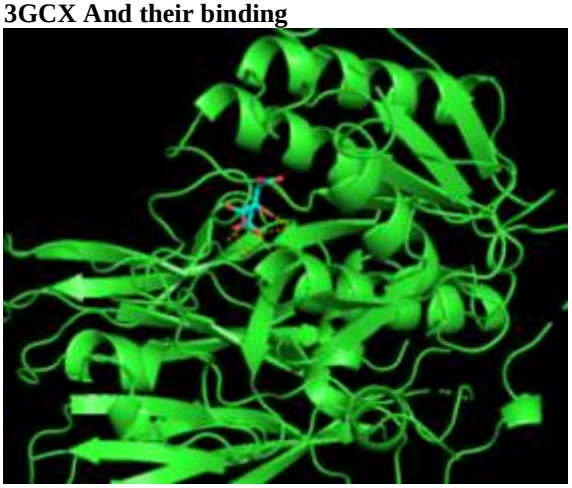
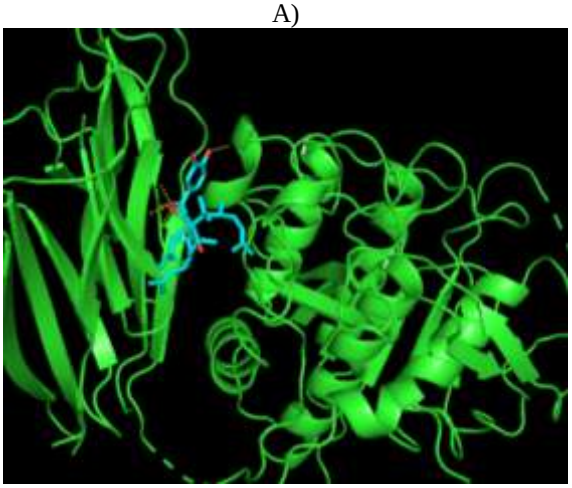


C)

Compounds docked against 3GCX And their binding



A)



B)

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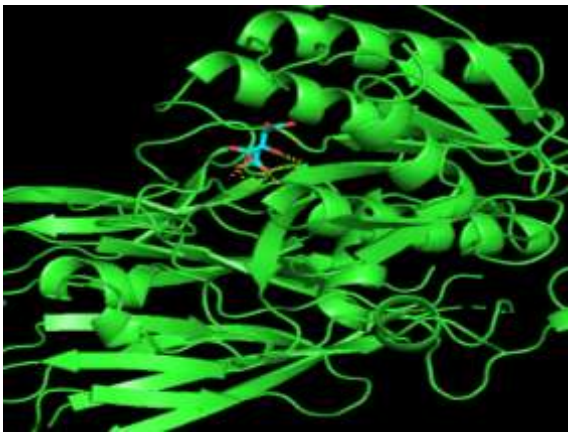

C)

Drug likeness:[19]

All the 3 molecules were subjected to in silico evaluation using **Molinspiration Cheminformatics Software Engine Vs2018.10** to evaluate drug-likeness. It can be accessed online for calculation of important molecular properties such as partition coefficient, binding with human serum albumin, the number of hydrogen bond donors and acceptors for the most important drug targets like GPCR ligands, kinase inhibitors, ion channel modulators and nuclear receptors. Drug likeness parameters are tabulated in Table 2.

Sr. No.	Compound	miLogP	TPSA	Molecular Weight(gm)	No. H Donate	No. H Accept	Volume	Rule of Five
1	Garcinol	6.73	111.90	618.86	6	6	624.15	2
2	Isogarcinol	6.78	100.90	618.86	6	2	619.61	2
3	Hydroxy-citric Acid	-2.90	152.35	208.12	8	5	159.81	0

From the table,

miLogP - It is used to predict the Partition co-efficient of the molecules. The values are normally in the range between -2.0 to 6.5.

TPSA (Total Polar Surface Area)- Molecular polar surface area (PSA) is a very useful parameter for the prediction of drug transport properties. Polar surface area is defined as a sum of surfaces of polar atoms (usually oxygens, nitrogens and attached hydrogens) in a molecule. This parameter has been shown to correlate very well with the human intestinal absorption, monolayers permeability, and blood-brain barrier penetration.

Molecular volume- Molecular volume determines transport characteristics of molecules, such as intestinal absorption or blood-brain barrier penetration. Volume is therefore often used in QSAR studies to model molecular properties and biological activity

Rule of 5 - Lipinski's rule said that molecules should possess MW<500, donor HB≤5, accept HB≤10, miLogP<5. Molecules that satisfy this rule are considered drug-like.

- **log P (partition coefficient) and log D (diffusion coefficient)**

Once the molecules were docked, then they were visualized for interactions, score and some other parameters like log P value and ionization value. There were interactions like hydrogen bonding, hydrophobic interaction, Van der Waal's interaction between the receptor and the ligand.

Pa Value: Probability to be active compounds
Pi Value: Probability to be inactive compounds

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1) Garcinol

value		Pass Prediction
Pa	**Pi**	**Activity**
0.864	0.003	Antioxidant
0.861	0.005	Anti-inflammatory
0.846	0.005	Apoptosis Agonist
0.417	0.026	Antibacterial
0.247	0.011	Lipoxygenase Inhibitor

2) Isogarcinol

value		Pass Prediction
Pa	**Pi**	**Activity**
0.896	0.004	Apoptosis Agonist
0.842	0.005	Anti-inflammatory
0.526	0.009	Free Radical Scavenger
0.480	0.007	Antioxidant
0.480	0.019	Lipid Peroxidase Inhibitor
0.459	0.043	Antiviral
0.386	0.034	Antibacterial
0.303	0.075	Antihypertensive

value		Pass Prediction
Pa	**Pi**	**Activity**
0.971	0.001	ATP Citrate Lipase Inhibitor
0.945	0.003	GPCR Kinase Receptor Inhibitor
0.871	0.004	Lipid Metabolism Regulator
0.811	0.015	Sugar Phosphate Inhibitor
0.804	0.012	NADPH Peroxidase inhibitor

3) Hydroxy-citric Acid

III. CONCLUSION

In silico studies like toxicity, docking and drug-likeness were performed for establishing safety and identifying the mechanism of action of some of the selected molecules which have been isolated from Garcinia Indica Leaves. Toxicity screening was done in silico using **OSIRIS** property explorer. Garcinol, Isogarcinol and Hydroxy-citric Acid were found to be non-toxic. In docking these compounds having the good docking score

It is also concluded from in silico studies of isolated compounds of Garcinia Indica Leaves, against 3GCX confirming the activity through its docking score. Most of the molecules are safe and effective. So, the molecules can be docked with other target proteins on overall perspective on the mechanism of action of these isolated compounds.

CONFLICT OF INTERESTS

The author declares no conflict of interest.

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