RESEARCH PAPER

In-silico evaluation of therapeutic effect of Protosappanin-A compound of Caesalpinia sappan L. (Pathimugham) on HMG-CoA reductase to reduce cholesterol levels in humans

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Abstract

In this study we evaluated role of Protosappanin-A, compound from ayurvedic plant *Caesalpinia sappan* known to be used for variety of health benefits. In this study Protosappanin-A compound docked to HMG-CoA reductase to check inhibition of the enzyme, which inturn will reduce the cholesterol levels in humans. Structure of Protosappanin-A was retrieved from Pubchem database, its chemical ID is 128001. The structure of HMG-CoA reductase was retrieved from RCSB-PDB database, its PDB ID is 1t02. ADME analysis was performed by deploying SwissADME server to primarily check Lipinski's violation, then Molecular docking and MD simulations were performed to explore the interaction between HMG CoA reductase and Protosappanin-A. It was noted that -39Kcal/mol binding energy with stable interaction patterns were present in between Protosappanin-A and HMG-CoA reductase. RMSD value was also found to be under 0.3 nm for the docked complex during simulation time span of 50ns.

Keywords: HMG-CoA reductase; MD Simulations; Molecular Docking; Pathimugham; Protosappanin-A

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Introduction

Since the middle of the 1990s, statins have been the primary evidence-based treatment for people with hypercholesterolemia. Statins have a track record of lowering the risk of cardiovascular disease (CVD), the recurrence of CVD events in people with ischemic heart disease (IHD), and also in people with genetically inherited familial hypercholesterolaemia (FH), whether or not they have established CVD. Although most patients tolerate and respond well to statin therapy, a significant proportion of patients are either unable to tolerate statin therapy or have not reached therapeutic total cholesterol (TC) and low-density lipoprotein-cholesterol (LDL-C) targets, leaving them at high risk of a cardiovascular event **(Ying et al., 2022)**. The initial stage of reverse cholesterol transport (RCT), cholesterol efflux from macrophages, is crucial for the prevention of atherosclerosis. Recent observational studies suggest that, independent of conventional cardiovascular risk factors like levels of low-density lipoprotein (LDL) and high-density lipoprotein (HDL) cholesterol, the ability of plasma to affect cholesterol efflux is inversely related to atherosclerotic cardiovascular disease (ASCVD). The ability of plasma acceptors to take up cholesterol produced from cells via various receptor-mediated mechanisms, such as ATP-binding cassette transporter A1 (ABCA1), ABCG1, scavenger receptor class B type I (SR-BI), and unspecific passive diffusion, is known as cholesterol efflux capacity (CEC). Through interactions with cellular receptors, lipid transfer proteins, lipases, and apolipoprotein (apo) B-100-containing lipoproteins, HDL plays a crucial part in RCT. With the use of such an integrated system, cholesterol can be transported back to the liver, or RCT, from peripheral cells like macrophages and foam cells. The most well-known method through which HDL prevents atherogenesis is RCT **(Ying et al., 2022)**. The precise methods or factors influencing cholesterol efflux are complicated; however they could be impacted by the last RCT phases. These include the availability of apoB-100-containing lipoproteins to accept cholesteryl esters from HDL particles, which allows the plasma system to maintain the capacity of HDL to take up free cholesterol in HDL particles constant. Because of its capacity to accelerate

the degradation of the LDL receptor, proprotein convertase subtilisin/kexin type 9 (PCSK9), a secretory protease that is mostly produced in the liver, is a crucial regulator of the metabolism of apoB-100-containing lipoproteins **(Ying et al., 2022)**. It has been repeatedly demonstrated that PCSK9 inhibition with monoclonal antibodies (mAbs), such as evolocumab and alirocumab, significantly lowers plasma concentrations of apoB-100-containing lipoproteins, such as very-low-density lipoprotein (VLDL), intermediate-density lipoprotein (IDL), LDL, and lipoprotein-A, with a modestly significant increase in HDL Statins significantly lower plasma concentrations of apoB-100 and LDL cholesterol while having little effect on HDL cholesterol levels because they inhibit 3-hydroxy-3-methylglutaryl-coenzyme A reductase, which stimulates LDL receptor activity for hepatic clearance of apoB-100-containing lipoproteins. In recent studies it was found that excessive use of statins results colorectal cancer **(Zhang et al., 2022)**; severe cognitive impairment in patients **(Alsubaie et al., 2022)**. In this study we evaluated role of Protosappanin-A, compound from ayurvedic plant *Caesalpinia sappan* known to be used for variety of health benefits. In this study Protosappanin-A compound docked to HMG-CoA reductase to check inhibition of the enzyme, which inturn will reduce the cholesterol levels in humans.

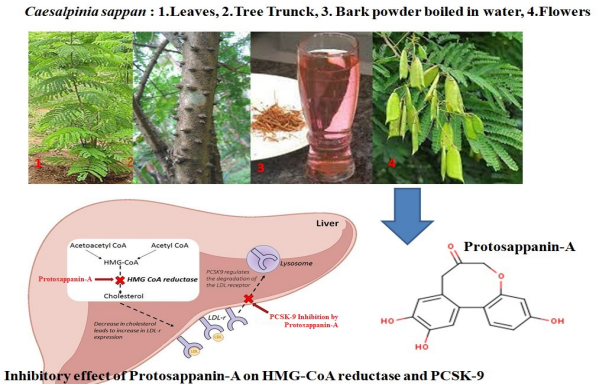

Figure 1. Protosappanin-A inhibiting HMG-CoA reductase and PCSK-9: A potential candidate for lowering blood Cholesterol level

Methodology

Structure retrieval

Structure of Protosappanin-A was retrieved from Pubchem database, its chemical ID is 128001. The structure of HMG-CoA reductase was retrieved from RCSB-PDB database, its PDB ID is 1t02.

ADME analysis

ADME analysis was performed by deploying SwissADME server **(Daina et al., 2017)** for phytochemical Protosappanin-A. Here we also analysed Lipinski violations, as we know The Lipinski Rule of Five (RO5), also referred to as Pfizer's Rule of Five or simply the Rule of Five (RO5), is a general guideline used to assess the druglikeness of a chemical compound or to determine whether it possesses chemical and physical characteristics that would likely make it an orally active drug in humans. Christopher A. Lipinski developed the rule in 1997 based on his observation that the majority of drugs taken by mouth are made up of small, moderately lipophilic molecules. According to Lipinski's rule **(Lipinski et al., 2012)**, an orally active medication generally has no more than one violation of the following standards:

- five maximum hydrogen bond donors (the total number of nitrogen–hydrogen and oxygen–hydrogen bonds)
- Ten maximum hydrogen bond acceptors (all nitrogen or oxygen atoms)
- a molecular weight of under 500 daltons
- octanol-water partition coefficient (log P) determined to be no more than 5

Molecular Docking Analysis

Docking analysis was performed by Autodock vina dependent server Webina 1.0.3 **(Kochnev et al., 2020)**. Here HMG-CoA reductase was docked with Protosappanin-A. Binding pocket was analyzed for the docked complexes to reveal binding energy and interaction patterns.

MD Simulation

Molecular dynamics for both the complexes was performed by deploying Webgro server **(Bekker et al., 1993; Abraham et al., 2015)** (https://simlab.uams.edu/). Here we selected the ligand (Protosappanin-A) and HMG-CoA reductase as a receptor, and set the parameters for molecular dynamics simulation analysis. The GROMOS96 43a1 force field, SPC water model, tetrahederal box type was selected with 0.15M NaCl. NVT/NPT equilibration with 310K temperature, 1 bar pressure, Leap-frog MD-indicator was set up for 50ns time span.

Results

ADME Analysis of Protosappanin-A

ADME analysis for Protosappanin-A indicates that this compound doesn't violate the Lipinski rule, and could be considered as good drug candidate. All other

informative analysis report is mentioned in **Table1** and **Figure 2**. This provides a direction that the compound Protosappanin-A completely show leadlikeness, and can be considered for further analysis via molecular docking.

Table 1. ADME analysis report of Protosappanin-A retrieved from Swiss-ADME

Molecule ADME Analysis	**Protosappanin-A**
Canonical SMILES	O=C1COc2cc(O)ccc2c2c(C1)cc(O)c(c2)O
Formula	C15H12O5
MW	272.25
#Heavy atoms	20
#Aromatic heavy atoms	12
Fraction Csp3	0.13
#Rotatable bonds	0
#H-bond acceptors	5
#H-bond donors	3
MR	72.3
TPSA	86.99
iLOGP	1.37
Consensus Log P	1.65
ESOL Log S	-3.21
ESOL Class	Soluble
Ali Log S	-3.42
Ali Class	Soluble
GI absorption	High
BBB permeant	No
Pgp substrate	Yes
CYP1A2 inhibitor	Yes
CYP2C19 inhibitor	No
CYP2C9 inhibitor	No
CYP2D6 inhibitor	No
CYP3A4 inhibitor	Yes
log Kp (cm/s)	-6.56
Lipinski #violations	0
Ghose #violations	0
Veber #violations	0
Egan #violations	0
Muegge #violations	0
Bioavailability	0.55
Score	
PAINS #alerts	1
Brenk #alerts	1
Leadlikeness #violations	0
Synthetic Accessibility	2.73

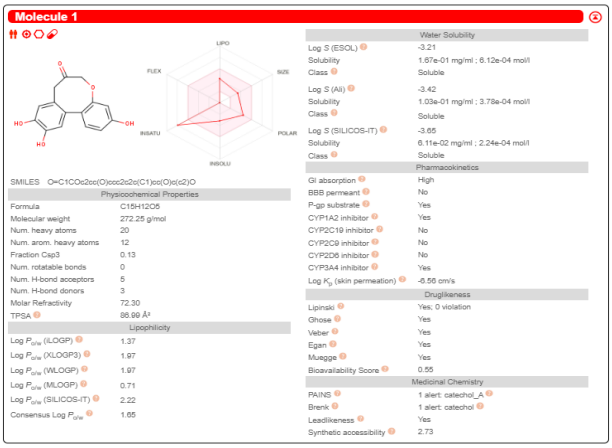

Figure 2. ADME Analysis of Protosappanin-A

Molecular Docking Analysis

Molecular docking was conducted between Protosappanin-A and 1t01 cleaned HMG COA reductase receptor. Here the box centre coordinates were X=86.66 Å, Y=122.03 Å, Z=127.25 Å; and the selected box size was x=18.66Å, y=14.42Å, and z=16.74Å. Molecular docking reveals that binding energy for the complex was -39.4 Kcal/ mol. Inhibition coefficient for this molecular docked complex was calculated by using notation: Inhibition constant (Ki) = exp (deltaG × 1000)/(Rcal × TK), where deltaG is the docking energy, Rcal is 1.98719, and TK is 298.15 **(Iman et al., 2015)**. The inhibition coefficient value was found to be 1.316488147 $\times 10^{-29}$ µM. The ligand atoms of Protosappanin-A shows hydrophobic interaction with B-chain of HMG-CoA reductase enzyme as shown in **Figure 3**. Such interaction patterns were checked by using Ligplot ver. 2.2 tool. Standard output.txt was retrieved (see **Figure 4**) for Standard output of Autodock Vina after performing molecular docking between HMG-CoA reductase and Protosappanin-A, which indicates finalized model.

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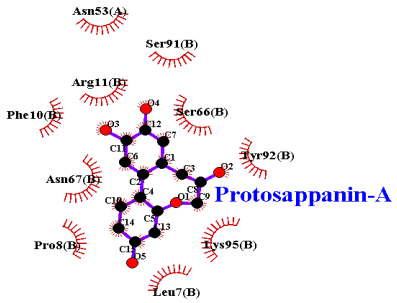

Figure 3. HMG CoA reductase and Protosappanin-A docked complex interaction view in Ligplot tool.

```
##########################################################
# If you used AutoDock Vina in your work, please cite:
#
# O. Trott, A. J. Olson,
# AutoDock Vina: improving the speed and accuracy of dockin
# with a new scoring function, efficient optimization and
# multithreading, Journal of Computational Chemistry 31 (20
# 455-461
#
# DOI 10.1002/jcc.21334
#
# Please see http://vina.scripps.edu for more information.
##########################################################

Output will be /ligand_out.pdbqt
Reading input ... done.
Setting up the scoring function ... done.
Analyzing the binding site ... done.
Using random seed: 1437244178
Performing search ...
0%   10   20   30   40   50   60   70   80   90   100%
|----|----|----|----|----|----|----|----|----|----|
**************************************************
done.
Refining results ... done.

mode |   affinity | dist from best mode
     | (kcal/mol) | rmsd l.b.| rmsd u.b.
-----+------------+----------+----------
   1         39.4      0.000      0.000
Writing output ... done.
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Figure 4. Standard output of Autodock Vina after performing molecular docking between HMG-CoA reductase and Protosappanin-A

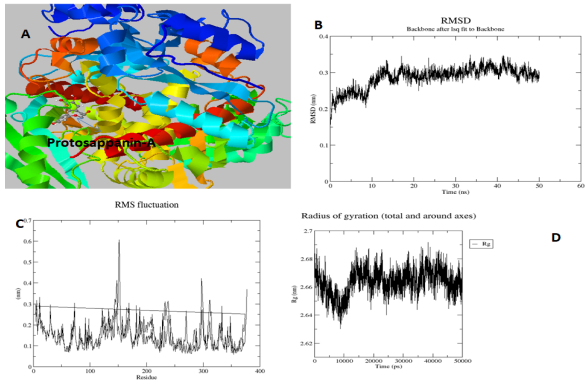

Figure 5. MD Simulation results of HMG-CoA reductase and Protosappanin-A: **A)** Docked complex , **B)** RMSD Plot, **C)** RMSF Plot, **D)** Radius of gyration Plot

MD Simulation

Molecular dynamics for both the complexes was performed by deploying Webgro server **(Bekker et al., 1993; Abraham et al., 2015)** (https://simlab.uams.edu/). Here we selected the ligand (Protosappanin-A) and HMG-CoA reductase as a receptor, and set the parameters for molecular dynamics simulation analysis. The GROMOS96 43a1 force field, SPC water model, tetrahederal box type was selected with 0.15M NaCl. NVT/NPT equilibration with 310K temperature, 1 bar pressure, Leap-frog MD-indicator was set up for 50ns time span. The results indicate clearly that RMSD(Root mean square deviation) for the Protein-ligand interaction was below 0.3nm, RMSF was also estimated in the range of 0.3nm to max of 0.6nm (see **Figure 5**). Hence, we can justify that this interaction is stable between Protosappanin-A and HMG-CoA reductase.

Discussion

The main cause of mortality around the globe is cardiovascular disease. Reducing mortality and slowing the development of cardiovascular organ damage are the long-term goals of treatment for cardiovascular disease. Regaining cardiac function and lowering risk factors including hyperglycemia and dyslipidemia are the main goals of current treatments. But oxidative stress and inflammation are significant contributors to further harm to cardiovascular organs. The blooming tree *Caesalpinia sappan* (Fabaceae), which is indigenous to tropical Asia, possesses anti-inflammatory and antioxidant effects **(Syamsunarno et al., 2021)**. In an effort to legitimise the plant *Caesalpinia decapetala* (Roth) Alston (*Caesalpiniaceae*), the antioxidant properties of the methanol extracts from the wood and pericarp were evaluated. In addition to its capacity to suppress lipid peroxidation, the plant's capacity to scavenge DPPH, superoxide, and nitric oxide radicals has been used to study its antioxidant activity. As measured by the DPPH, superoxide radical, nitric oxide radical, total phenols, flavonoids, and total flavonols, the pericarp's antioxidant activity and phenolic content were higher than those of the wood **(Gallego et al., 2017)**. In current study we used latest tools of molecular docking and MD-Simulation to explore the interaction of Protosappanin-A compound from *Caesalpinia sappan* towards HMG CoA reductase, this study revealed that Protosappanin-A inhibits the action of HMG CoA reductase and in turn reduces the level of cholesterol in the case of hypercholestremia. This study

will open future scope of MD Simulations in analysing drug or ligand molecules interaction towards enzymatic complexes. Artificial neural networks, Big data analysis, immunoinformatics and chemi-informatics approaches are recently much explored after the advent of corona **(Joshi et al., 2022A; Joshi et al., 2021; Joshi et al., 2022B; Joshi et al., 2022C)**; and these studies assisted scientific community to rapidly design best treatments by exploring native ayurvedic flora, and our here our study explore a traditional medicinal plant pathimugham *Caesalpinia sappan* for the treatment of hypercholesterolemia.

Conclusion

This study shows Protosappanin-A compound of *Caesalpinia sappan* extract can lower the cholesterol level, as it inhibits the HMG CoA reductase enzyme. Also binding energy between Protosappanin-A and HMG CoA reductase was found to be -39.4 Kcal/mol, and during MD simulation analysis it was noted that there was stable protein-ligand interaction.

CONFLICT OF INTEREST:

The authors have no conflicts of interest.

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