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ORIGINAL ARTICLE **OPEN ACCESS**

In silico Investigation of Mode of Action of Certain Isolated Compounds of Tephrosia Species as Antihyperlipidemic Agents

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ABSTRACT
Polyphenols and flavonoids have a significant role in conquering hyperlipidemia, one of the major risk factors causing cardiovascular diseases (CVDs). The molecular docking of few selective metabolites of Tephrosia tinctoria and Tephrosia villosa with HMG-CoA reductase (1DQ8), lipoprotein lipase (6E7K), lecithin cholesterol acetyl transferase (4X96), compared with Atorvastatin and Simvastatin. Cluster of Ligands were prepared using BIOVIA Discovery studio and the molecular docking was carried out using PyRX software (Autodock-Vina). Based on docking studies, tephcalostan, 12a-dehydro-6-hydroxysumatrol, 5,7-di-O-prenylbiochanin A and Villinol bind well to the active site of HMGR and reduce its catalytic activity by structure change. Similarly, tephcalostan, 12a-dehydro-6-hydroxysumatrol, 5,7-di-O-prenylbiochanin A, lupenone, and lupeol bind to and enhance the enzymatic activity of LPL, and dehydroeguelin, tephcalostan, lupenone, and lupeol stimulate the activity. enzyme of LCAT by binding to its catalytic site.The Protein Ligand Complex was built using PyMol. About 18 isolated phytoconstituents were evaluated by submitting to ADMET Predictor calculations with default settings in SwissADME (http://www.swissadme.ch/). The binding affinity of many compounds was better than the standard and many compounds had some common amino acids interaction with H-bonding. All the 18 compounds have good GI absorption which ranges from 42.32% to 100% and shows good distribution in all the body fluids. These compounds were metabolized in liver by CYP450 isozymes and the total clearance values good for all the compounds. All the compounds are nontoxic except 3 compounds which show hepatotoxicity. Hence, this study concludes that a few of the polyphenolic and flavonoids from Tephrosia villosa and Tephrosia tinctoria can be considered as antihyperlipidemic agent in treating hyperlipidemia.
Keywords: *Polyphenols, 1DQ8, 6E7K, 4X96, ADME/T, Anti-hyperlipidemic.*

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INTRODUCTION

Hyperlipidemia is considered one of the major risk factors for cardiovascular diseases (CVDs), including atherosclerosis, coronary heart disease, and hypertension. Several studies have reported that these diseases are the leading cause of death and disability worldwide population. Recent studies have reported that high cholesterol is present in 25-30% of urban and 15-20% rural subjects. This prevalence is lower than high-income countries [1, 2]. Based on the data analysis, elevated lipoprotein (a) levels are a major cause for atherosclerotic cardiovascular disease which is highly dependent on genetic factors than dietary or behavioral factors or therapeutic lifestyle intervention [3,4]. Additionally, the relationship between dietary cholesterol and these diseases has been a topic of extreme research. Admittedly, the consumption of high diet would have the impact on lipid metabolism and changes in serum lipid profile with increasing total cholesterol, triglycerides and low-density lipoprotein (LDL)-cholesterol and decrease of high-density lipoprotein (HDL)-cholesterol. [5] Recently, a clinical study on middle-aged men suggested the crucial relevance of intermediate-density lipoprotein cholesterol (IDL-C) for the risk of coronary heart disease and stroke. [6] Another clinical study suggests that remnant lipoproteins, lipoprotein (a), and inflammation are related to the risk of atherosclerotic cardiovascular disease who is already with any statin therapy. [7]

Innumerable animal experiments and clinical trials have demonstrated that by reducing the levels of atherogenic lipids the cardiovascular morbidity and mortality can be decreased. [8] The current treatment options for hyperlipidemia mainly include statins, fibrate, nicotinic acid, bile acid binding resins, and the same are associated with significant number of side effects such as gastrointestinal events, headache, nausea, diarrhea and vomiting. [9] One of the preclinical studies showed a remarkable

reduction in the oxidative and inflammatory stress to the liver by clofibrate use in combination with sesamol, a natural phenolic compound. [10] There are many natural plants that have hypolipidemic effects. A study on *Solidago virgaurea* hydroalcoholic extract rich in flavonoids and polyphenols, showed a remarkable hypolipidemic effect in type I diabetic rats. [11] Similarly polyphenolic content of a plant *Lippia citriodora* showed a significant effect on lipid profile in a recent preclinical and clinical study. [12, 13] Also natural phenolic compounds like Tyrosol, Methylophiopogonanone A (MO-A) and n-octadecanyl-O-α-D-glucopyranosyl (6′→1″)-O-α-D-glucopyranoside were considered to be a candidate molecule for dyslipidemia condition. [14-16] Thus, there prevails a discontented need for the efficacious and safe remedy for hyperlipidemia. A tremendous upsurge has been witnessed in the field of herbal preparations in both developed and developing countries in the last few years because of their natural extraction and lesser side effects. About 51% of the approved drugs is adherent to the natural origin. [17,18] The present study was focused on *Tephrosia* genus plant constituents for their antihyperlipidemic efficacy.

The completion of the human genome project has resulted in an increasing number of new therapeutic targets for drug discovery. Bioinformatics necessitates the organization of data generated from experiments into databases, the development of new algorithms and software, utilizing it for the explication and scrutinization of data. Virtual screening can be categorized into ligand-based and structure-based methods. [19] The ligand-based methods, for instance pharmacophore modeling and quantitative structure activity relationship (QSAR) methods can be made use of when a set of active ligand molecules is known, and little or no structural details are available for targets. For structure-based drug design, molecular docking is the most common method which has been widely used ever since the early 1980s. [19] The prime focus of molecular docking is to use the preferred orientation of the compound relative to the receptor to predict the binding strength and affinity of the receptor and ligand thus permitting to characterize the behavior of small molecules in the binding site of target proteins as well as to annotate the fundamental biochemical processes. [20] The two basic steps in docking involve foreseeing the ligand conformation as well as its position and orientation within these sites and evaluation of the binding affinity. In pharmaceutical research, programs based on different algorithms were developed to perform molecular docking studies, contributing docking as an increasingly important tool [19, 21].

MATERIAL AND METHODS

Molecular Docking protocol

Docking studies were carried out to analyze the different types of biomolecular interactions and ligand receptor binding affinities. The docking studies were carried out by means of Autodock vina, Biovia Discovery Studio 2020, PyRX, and PyMOL. The docking study was performed on crystal structure of complex of the catalytic portion of human HMG-CoA reductase with HMG and COA (pdb id: 1DQ8), Lipoprotein Lipase (pdb id: 6E7K), Lecithin cholesterol Acetyl transferase (pdb id: 4X96). The computational work was performed on a HP 15s-eq0132au Laptop running on AMD Ryzen 7 3700U processor.

Protein preparation

The crystal structure of complex of the catalytic portion of human HMG-CoA reductase (HMGR) with HMG-CoA, Lipoprotein Lipase, Lecithin cholesterol Acetyl transferase was retrieved from the RCSB Protein Data Bank, PDB entry codes: 1DQ8, 6E7K and 4X96 respectively.

Ligand preparation

The structures of a few of the compounds were drawn in Chemdraw and a few were downloaded from RCSB PDB (https://www.rcsb.org) and uploaded in BIOVIA Discovery Studio Visualizer-2020. Ligand minimization was done and using small molecule wizard in 'SMALL MOLECULE' wizard in BIOVIA Discovery Studio Visualizer-2020, ligand preparation was carried out.

Molecular Docking

To reduce false positives and to identify the perfect orientation of ligand within the active site of protein, docking study acquires its importance. Docking was done using PyRx-Virtual Screening Tool. Converted all ligands to pdbqt in PyRx-Virtual Screening Tool and selected those as ligands in Vina wizard. Loaded the prepared proteins 1DQ8, 6E7K and 4X96 respectively into the PyRx-Virtual Screening Tool and selected it as macro molecule. The grid size in the protein molecule was selected based on the data acquired from Cast-p server. Calculation of interaction energy (interaction between ligand and receptor), amino acid involved in binding was carried out.

Drug-like properties

All the selected phytoconstituents were assessed for its drug likeliness by using Molinspiration server which anticipate the molecular weight (MW), hydrogen bond acceptors (HBA) and donors (HBD), number of rotatable bonds and lipid water partition coefficient (logP) thus predicts the Lipinski's rule of five. [21]

ADME/T Studies

All 18 isolated phytochemical compounds were evaluated for their ADME parameters sketched using Chemdraw and downloaded from pubchem and were saved as SMILES. These files were submitted to ADMET Predictor calculations with default settings in SwissADME (http://www.swissadme.ch/) to predict their potential pharmacokinetic properties and toxicity.

RESULT AND DISCUSSION

Molecular Docking Studies

Lipids and various lipoproteins are independent hazards for cardiovascular disorders (CVD). Hence, lipid profile, a pivotal factor in the progression and pathogenesis of CVD. Hypercholesterolemia and/or hypertriglyceridemia are significant influencing factors in the emerging CVD. [22] In current research, molecular docking remains the strong domain in the field of computer-based drug design. The hydrogen bonding and hydrophobic interactions in protein-ligand complexes play a predominant role in assessing their binding affinity and stability. [16]

The molecular docking studies of the various isolated compounds from Tephrosia tinctoria and Tephrosia villosa with HMG-CoA reductase (PDB ID: 1DQ8), lipoprotein lipase (PDB ID: 6E7K) and lecithin cholesterol acyl transferase enzymes (PDB ID: 4X96). The docking of all the compounds with the active sites of 1DQ8, 6E7K and 4X96 proteins exhibited the hydrogen bonding with certain amino acids similar to the standard drug Simvastatin and Atorvastatin. The binding affinity of many compounds was better than Simvastatin in all the 3 proteins (1DQ8, 6E7K and 4X96).

In molecular docking, lower the binge energy in protein-ligand complexes indicates higher the stability of complexes. [23] The binding scores of ligands were between -7.1 to -8.6 kcal\mol where the binding scores of Simvastatin and Atorvastatin was -7.5 and -7.4 kcal\mol respectively with the protein 1DQ8. Also, few phenolic compounds like 12a-dehydro-6-hydroxysumatrol, 5,7-di-O-prenylbiochanin A, Tephcalostan, Lupinifolin & Villinol showed good binding to the active site of HMGR by H-bonding and other interactions with amino acid residues with lower binding energy that is higher than Simvastatin and Atorvastatin which reinforce the protein-ligand complex stability (Table 1, Figure 1). Hence this interaction may be the probable mechanism as its competitive inhibition of HMGR active site. Earlier studies suggested that this inhibition reduces the cholesterol in endoplasmic reticulum and elevates sterol regulatory element-binding proteins (SREBPs) ferry to Golgi apparatus of the cell. The SREBPs induces discharge of VLDL and LDL from plasma by LDL receptor gene expression. [24, 25] The results obtained were similar to the docking study on phytochemicals like phenolic compounds and sterols present in ethanolic extract of Prosopis cineraria pods, showed HMG – CoA reductase inhibitory activity. [26]

Table 1. Docking score and H-Bond interactions of ligands with HMGR (1DQ8)

Ligand	***Binding Affinity (kcal\mol)***	***Binding Residues***
12a-dehydro-6-hydroxysumatrol	-8.5	Val D: 540, Leu C: 499, Asn D: 567, Coa D: 103, Pro C: 477, His C: 475, Lys C: 474, Ile C: 476, Ala C: 478
5,7-di-O-prenylbiochanin A	-8.3	Asn C: 472, Lys C: 502, Ala C: 478, Asn D: 567, Gly D: 542, Arg D: 571
7-O-methylglabranin	-7.4	Pro D: 513, Pro C: 813, Leu C: 812, Tyr C: 533, Tyr D: 533, Asp C: 516
Betulinic acid	-7.8	Pro C: 477, Tyr C: 479, Ala C: 478, Val C: 471
Dehydrodeguelin	-8.2	His C: 475, Pro C: 477, Coa D: 103, Asn D: 567, Gly D: 542, Leu C: 499, Val D: 540, Gln D: 552, Leu C: 498, Arg C: 495, Lys C: 502, Ala C: 478
Dehydrorotenone	-7.8	Coa D: 103, Has C: 475, Lys C: 474, Pro C: 477, Ala C: 478, Val D: 540, Leu C: 499
Flemichapparin B	-7.6	Lys C: 474, His C: 475, Ala C: 478, Asn D: 567, Pro C: 477
Lupenone	-7.5	Asn C: 529, Lys C: 474
Lupeol	-7.4	Coa D: 103, Glu C: 482
Lupinifolin	-8.6	Val C: 471, Leu C: 498, Ala C: 478, Pro C: 477, Coa D: 103, Asn D: 567, Val D: 540, Arg C: 495, Leu C: 499
Rotenone	-7.5	Lys C: 474, His C: 475, Pro C: 477, Ala C: 478, Asn C: 529, Val D: 540, Lys C: 502, Leu C: 499
Stigmasterol	-7.4	Tyr D: 511, Leu C: 812, Pro D: 513, Pro C: 813, Arg D: 515
Tephcalostan	-9.1	Lys C: 474, His C: 475, Ala C: 478, Lys C: 502, Val D: 540, Leu C: 499, Pro C: 477
Tephrinone	-7.7	Tyr C: 533, Tyr D: 533, Asp C: 516, Pro C: 813, Pro D: 513, Leu C: 812
Tephrowatsin_C	-7.2	Pro C: 813, Leu C: 812 , Pro D: 513, Tyr D: 533, Asp C: 516
Villinol	-8.1	His C: 475, Lys C: 474, Pro C: 477, Coa D: 103, Asn D: 567, Leu C: 499, Val D: 540, Ala C: 478
Villol	-7.6	Coa D: 103, Lys C: 474, Pro C: 477, Asn C: 472, Val C: 471, Lys C: 502, Lys C: 501, Leu C: 498
Villosin	-7.1	Asn D: 567, Asn C: 529, Pro C: 477
Simvastatin	-7.5	Pro C :477, Asn D: 567, Lys C: 502
Atorvastatin	-7.4	Asn C: 472, Lys C: 474, Asn B: 567, Coa B: 103, Gly D: 542, Asn C: 529

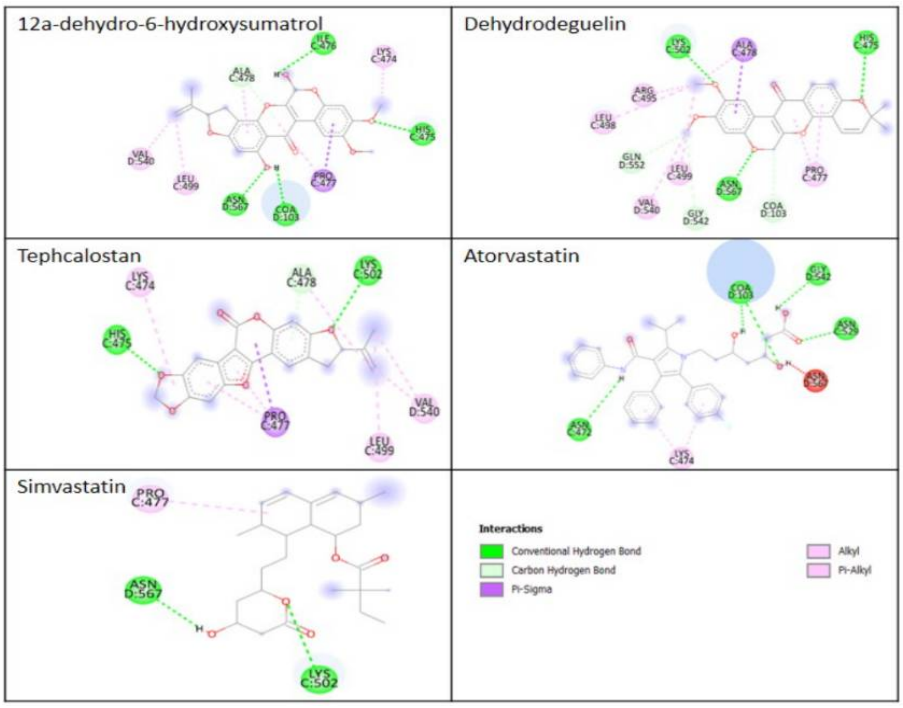

Figure 1. The 2D interactions of 12α-dehydro-6-hydroxysumatrol, Dehydrodeguelin, Tephcalostan, Simvastatin & Atorvastatin with HMGR (1DQ8)

Lipoprotein lipase (LPL), a key enzyme hydrolyzes triglycerides present in chylomicrons and VLDL-C in the plasma and alters the metabolism of lipoproteins which augment the depletion of hepatic lipoproteins. Hence increased levels of LPL are anti-atherogenic whereas decreased levels are atherogenic. [22] The binding scores of Ligands were between -7.7 to -9.6 kcal\mol where the binding scores of Simvastatin and Atorvastatin was -8.0 and -8.1 kcal\mol respectively with the protein 6E7K (Table 2, Figure 2). Hence, the binding affinity of a few ligands in the active site may potentiate the LPL activity leads to having anti-atherogenic properties.

Table 2. Docking score and H-Bond interactions of ligands with LPL (6E7K)

Ligand	Binding Affinity (kcal\mol)	Binding Residues
12a-dehydro-6-hydroxysumatrol	-9.3	Ala A: 158, Arg A: 192, Asp A: 234, Glu A: 62, His A: 241, Ile A: 194, Leu A:133, Lys A: 238, Pro A: 160, Ser A: 132, 193, Thr A: 56, 131, Trp A: 55, 64, Tyr A: 94, Val A: 57, 233, 237
5,7-di-O-prenylbiochanin A	-8.5	Arg A: 192, Asp A: 234, Glu A: 62, His A: 241, Ile A: 194, Lys A: 238, Pro A: 160, Ser A: 132, Thr A: 56, 58, Trp A: 55, Tyr A: 94, 131, Val A:57, 237
7-O-methylglabranin	-8.1	Ala A: 158, Asp A: 234, Gly A: 159, His A: 241, Ile A: 194, Lys A: 238, Pro A: 160, Ser A: 132, Thr A: 56, Trp A: 55, Tyr A: 94, Val A: 57, 237
Betulinic acid	-8.8	Asp A: 234, His A: 93, 241, Ile A: 194, Lys A: 238, Phe A: 185, Pro A: 95, 160, Trp A: 55, Tyr A: 94, Val A: 57, 233, 237
Dehydrodeguelin	-8.3	Asp A: 234, Glu A: 62, His A: 241, Ile A: 194, Lys A: 238, Pro A: 160, Ser A: 132, Trp A: 55, Tyr A: 94, 131, Val A: 233
Dehydrorotenone	-9	Arg A: 192, Glu A: 62, 242, His A: 241, Ile A: 194, Lys A: 238, Pro A: 160, Ser A: 132, Thr A: 56, Trp A: 55, 64, Tyr A: 131, Val A: 57, 237
Flemichapparin B	-8.6	Glu A: 62, His A: 241, Ile A: 194, Lys A: 238, Pro A: 160, Ser A: 132, Trp A: 55, Tyr A: 94, 131
Lupenone	-9.5	Asp A: 234, His A: 241, Ile A: 194, Lys A: 238, Pro A: 95, 160, Ser A: 132, Trp A: 55, Tyr A: 94, Val A: 57, 237
Lupeol	-9.3	Asp A: 234, Asn A: 161, His A: 93, 241, Ile A: 194, Lys A: 238, Phe A: 185, Pro A: 95, 160, Trp A: 55, Tyr A: 94, Val A: 57, 237
Lupinifolin	-9.6	Ala A: 158, Glu A: 62, Gly A: 159, His A: 241, Ile A: 194, Lys A: 238, Pro A: 160, Ser A: 132, Thr A: 56, Trp A: 55, Tyr A: 94, 131, Val A: 57, 237
Rotenone	-8.3	Arg A: 192, Asp A: 234, Asn A: 161, His A: 241, Ile A: 194, Lys A: 238, Pro A: 95, 160, Ser A: 132, Trp A: 55, Tyr A: 94, Val A: 237
Stigmasterol	-8.9	Asp A: 234, Glu A: 62, His A: 241, Ile A: 194, Lys A: 238, Pro A: 160, Ser A:

		132, Thr A: 56, Trp A: 55, 64, Tyr A: 94,131 Val A: 57
Tephcalostan	-9.4	Ala A: 158, Glu A: 62, Gly A: 159, His A: 241, Ile A: 194, Lys A: 238, Pro A: 160, Ser A: 132, Thr A: 56, Trp A: 55, 64, Tyr A: 94, 131, Val A: 57
Tephrinone	-8.7	Asp A: 234, His A: 241, Ile A: 194, Lys A: 238, Pro A: 160, Ser A: 132, Trp A: 55, Tyr A: 131, Val A: 57, 233, 237
Tephrowatsin C	-8.1	Ala A: 158, Glu A: 62, Gly A: 159, His A: 241, Lys A: 238, Pro A: 160, Ser A: 132, Trp A: 55, Tyr A: 94, 131,
Villinol	-8.5	Ala A: 158, Glu A: 62, 242, His A: 241, Ile A: 194, Leu A:133, Lys A: 238, Pro A: 160, Ser A: 132, Thr A: 56, Trp A: 55, 64, Tyr A: 94, 131, Val A: 237
Villol	-7.7	Glu A: 271, Ile A: 349, 366, Leu A:365, Lys A: 272, 304, Phe A: 351, 364, Pro A: 350, 354, Ser A: 363, Tyr A: 367
Villosin	-8.7	Glu A: 62, His A: 241, Ile A: 194, Lys A: 238, Pro A: 160, Thr A: 56, Trp A: 55, Tyr A: 94, 131, Val A: 57, 237
Simvastatin	-8.0	Asp A: 234, Glu A: 62, His A: 241, Ile A: 194, Lys A: 238, Ser A: 132, Thr A: 56, 58, Trp A: 55, Tyr A: 94, 131, Val A: 57, 233, 237
Atorvastatin	-8.1	Ala A: 158, Asp A: 234, Glu A: 62, Gly A: 59, 159, His A: 241, Ile A: 194, Lys A: 238, Met A: 60, Pro A: 160, Ser A: 132, Thr A: 56, 58, Trp A: 55, Tyr A: 94, 131, Val A: 57, 233, 237

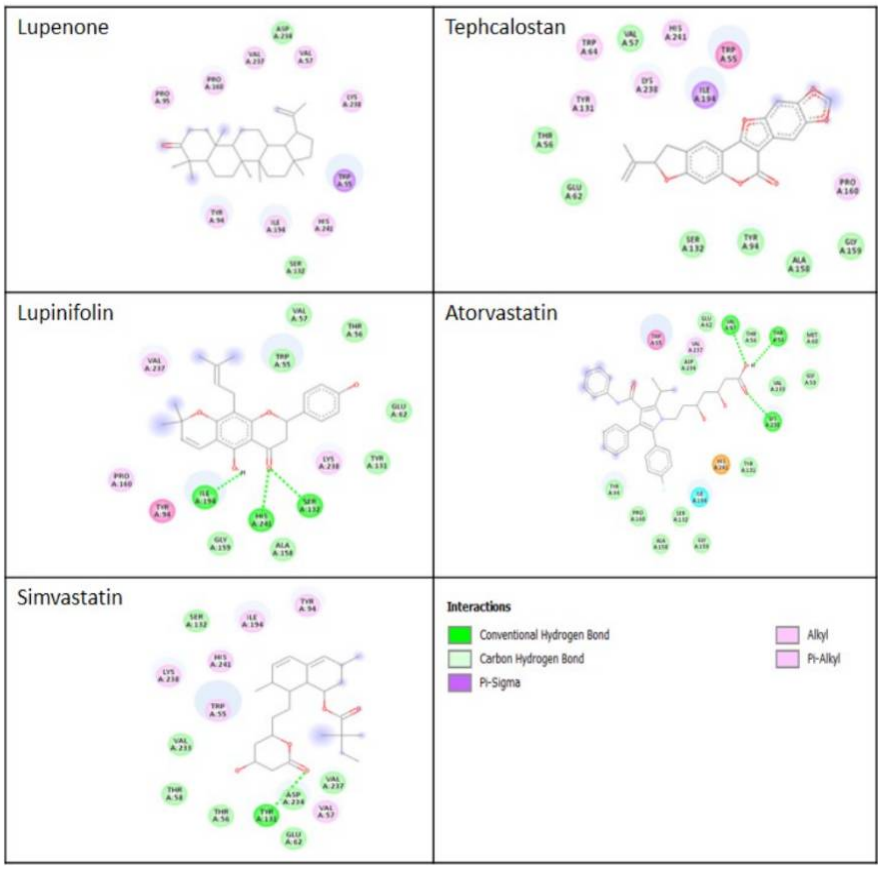

Figure 2. The 2D interactions of Lupenone, Tephcalostan, Lupinifolin, Simvastatin & Atorvastatin with LPL (6E7K)

The LCAT is an essential enzyme for HDL-cholesterol formation and maturation from tissue cholesterol by preferentially process the transfer of the sn-2 acyl group from lecithin molecule to cholesterol, forming a cholesteryl ester which segregates the hydrophobic core of the HDL molecule. Hence the impairment of LCAT will strengthen the atherosclerosis and reduce the HDL maturation. [27, 28] The binding scores of Ligands were between -7.5 to -8.9 kcal\mol where the binding scores of Simvastatin and Atorvastatin were -7.2 and -7.7 kcal\mol respectively with the protein 4X96 (Table 3, Figure 3). This indicates the potentiation of LCAT active sites may augment the cholesterol degradation by phenolic compounds.

Table 3. Docking score and H-Bond interactions of ligands with LCAT (4X96)

Ligand	Binding Affinity (kcal\mol)	Binding residues
5,7-di-o-prenylbiochanin A	-7.5	His A: 266, Glu A: 268, 269, Arg A: 60, 127, Val A: 61, Phe A: 267, Leu A: 203, 265, Thr A: 59, 226, 227, 228, Trp A: 126, Ile A: 56
7-O-methylglabranin	-7.7	Leu A: 219, 358, Ile A: 211, 213, Lys A: 220, 218, Glu A: 221, Gln A: 209, Asn A: 208, Phe A: 362, Pro A: 212
12a-dehydro-6-hydroxysumatrol	-7.9	Arg A: 79, Asn A: 72, Asp A: 125, Gln 132, 133, 134, 135, Ile A: 78, Leu A: 69, 128, Lys A: 139, Phe A: 83, Pro A: 123, Ser A: 71, Tyr A: 124, 136, Val A: 69, 80
Betulinic acid	-8.5	Asn A: 45, 58, Asp A: 43, Met A: 29, 46, Leu A: 20, 48, Cys A: 54, Gly A: 51, Ile A: 40, Trp A: 55, Tyr A: 31, Phe A: 38
Dehydroeguelin	-8.3	Arg A:60, 127, His A: 266, Leu A: 203, 265, Met A: 273, Phe A: 267, Thr A: 226, 227, 228, Val A: 61
Dehydrorotenone	-7.9	Arg A: 60, 127, Cys A: 11, His A: 266, Ile A: 56, Leu A: 203, Phe A: 267, Ser A: 229, Thr A: 59,227,228, Trp A: 126, Val A: 61
Flemichapparin_B	-7.8	Arg A:60, 127, His A: 266, Leu A: 203, 265, Phe A: 267, Thr A:226, 227, 228, Trp A: 126, Val A: 61
Lupenone	-8.6	Arg A:79, 127, Asp A: 125, Asn A: 72, Gln A: 77, Glu A: 135, Ile A: 78, Leu A: 128, Lys A: 139, Phe A: 83, Ser A: 71, Tyr A: 124, 136, Val A: 80
Lupeol	-9	Asp A: 43, Asn A: 45, 58, Cys A: 54, Gly A: 51, Ile A: 40, Leu A: 20, 48, Met A: 29, 49, Phe A: 38, Tyr A: 31, Trp A: 55
Lupinifolin	-8	Arg A: 60, 127, His A: 266, Glu A: 269, Leu A: 128, 203, 265, Phe A: 267, Thr A: 226, 227, 228, Val A: 61
Rotenone	-8	Arg A: 60, 127, His A: 266, Ile A: 56, Leu A: 203, Phe A: 267, Thr A: 226, 227, 228, Val A: 61
Stigmasterol	-8.5	Arg A: 60, 127, Ala A: 204, His A: 266, Ile A: 56, Leu A: 203, 265, Pro A: 312, Phe A: 267, Thr A: 226,227,228, Val A: 61
Tephcalostan	-8.9	Arg A: 60, 127, His A: 266, Leu A: 203, 265, Pro A: 312, Phe A: 267, Thr A: 59, 226, 227, 228, Val A: 61
Tephrinone	-7.9	Asn A: 58, Asp A: 43, Cys A: 54, Gly A: 51, Leu A: 20, 48, Met A: 29, 46, Phe A: 38, Thr A: 34, Tyr A: 31, Trp A: 55
Tephrowatsin C	-7.5	Asn A: 45, 58, Asp A: 43, Cys A: 54, Gly A: 51, Leu A: 48, Met A: 29, 46, Phe A: 38, Thr A: 34, Tyr A: 31, Trp A: 55, Val A: 52
Villinol	7.6	Arg A: 60, 127, Glu A: 269, His A: 266, Leu: 203, 265, Met A: 273, Thr A: 226, 227, 228, Val A: 61
Villol	-7.5	Arg A: 60, 127, Cys A: 11, Ile A: 56, Leu A: 203, Phe A: 267, Pro A: 230, Ser A: 229, Trp A: 126, Thr A: 59, 226, 227, 228, Val A: 61
Villosin	-8	Arg A: 60, 127, Ile A: 56, Leu A: 203, Met A: 273, Phe A: 267, Thr A: 59, 226, 227, 228, Val A: 61
Simvastain	-7.2	Asn A: 208, Cys A: 11, Glu A: 221, Gln A: 209 Ile A: 211, 213, 217, Leu A: 219, 358, Met A: 214, Pro A: 212, Ser A: 215, 216, Val A: 61
Atorvastatin	-7.7	Arg A: 79, 127, Asp A: 125, Asn A: 72, Gln A: 77, 132, 133, Glu A: 135, Ile A: 78, Leu A: 128, Lys A: 139, Pro A: 123, Ser A: 71, Try A: 124, 136, Val A: 70, 76, 80

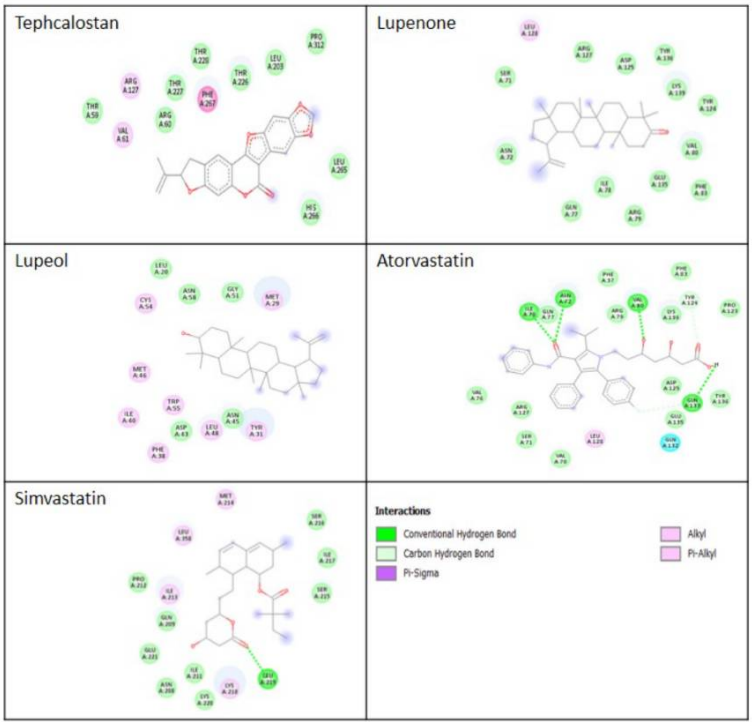

Figure 3. The 2D interactions of Lupenone, Lupeol, Tephcalostan, Simvastatin & Atorvastatin with LCAT (4X96)

A recent in vivo study of extracts of Thymus atlanticus leaves, contains more of phenolic constituents like Rosmarinic acid exhibited a significant antihyperlipidemic effect in hamsters with the expected mechanism of action by HMG – CoA reductase inhibitory activity and reduce intestinal cholesterol absorption. [29] Another study suggests that kaempferitrin, L-ascorbic acid, rutin, catechin, and isoquercetin present in hydroalcoholic extract from Rosa roxburghii shows antihyperlipidemic effect which strengthens the present study by exhibiting good binding affinity to all the proteins (1DQ8, 6E7K and 4X96) by the phenolic contents of Tephrosia tinctoria and Tephrosia villosa. [30]

Drug Likeliness

All the eighteen reported isolated compounds from Tephrosia tinctoria and Tephrosia villosa were analyzed for the various parameters of Lipinski's rule found to lie within the normal standard values. The compounds namely Lupenone, Lupinifolin, Betulinic acid and Stigmasterol were found to have violation in two parameters. The results are expressed in Table 4.

Table 4. Drugability studies of the ligands viz., parameters of Lipinski's rule of 5

Name of the Compound	MR (40-130)	logP (<5)	H-bond acceptors (<10)	H-bond donors (<5)	M.Wt (<500)
12a-dehydro-6-hydroxysumatrol	111.92	3.12	8	2	424.4
5,7-di-O-prenylbiochanin A	114.914	5.00	5	1	394.46
7-O-methylglabranin	80.51	2.79	5	1	300.31
Betulinic acid	136.91	7.09	3	2	456.71
Dehydrodeguelin	109.56	4.29	6	0	392.4
Dehydrorotenone	108.73	4.1	6	0	392.4
Flemichapparin B	78.657	3.58	5	0	296.27
Lupenone	134.18	8.23	1	0	424.71
Lupeol	135.14	8.02	1	1	426.7
Lupinifolin	117.39	4.96	5	2	406.47
Rotenone	106.15	3.7	6	0	394.42
Stigmasterol	132.75	7.8	1	1	412.7
Tephcalostan	99.12	4.3	6	0	362.33
Tephrinone	97.74	4.29	4	1	338.4
Tephrowatsin C	99.41	3.49	5	2	356.41
Villinol	116.65	3.78	8	1	438.43
Villol	110.38	1.72	9	3	442.42
Villosin	91.26	4.82	2	0	300.44

ADME/T Studies

In ADME/T properties, the absorption rate above 30% symbolizes good intestinal absorption. Similarly, the VDss (volume of distribution) is important for other pharmacokinetic properties. The value of VDss of compounds in brain (logBB) is < -1 and > 0.3 are considered as poorly and significantly crosses the BBB respectively. [31-33] In the present study, all the compounds from Tephrosia tinctoria and Tephrosia villosa evaluated showed good GI absorption which ranges from 42.32% to 100% and also shows good distribution in all the body fluids. These compounds were metabolized in liver by CYP450 isozymes and the total clearance values were good for all the compounds. The correlation between the rate of drug excretion and concentration of drug in the body is significantly altered by total clearance. [34-36] Also, the assessment of toxicity is essential due to its crucial role in drug selection. All the compounds were nontoxic except 3 compounds which showed hepatotoxicity. The reports retrieved from SwissADME were tabulated, analyzed and reported in Table 5.

Table 5.:ADME/T profile of ligands of Tephrosia tinctoria and Tephrosia villosa

Compounds	Ab	Db			Mt							Ex	Tx	
					Substrate		Inhibitor							
					CYP									
	IA (human)	VDss (human)	BBB Pr	CNS Pr	A	B	C	D	E	F	G	TC	AMES Tx	HT Tx
	% Ab	Log L/kg	Log BB	Log PS	Yes(Y)/ No(N)							LogmL/min/kg	Yes/ No	Yes/ No
12a-dehydro-6-hydroxysumatrol	96.471	0.57	-0.549	-3.015	N	N	N	N	Y	N	N	0.411	N	N
5,7-di-O-prenylbiochanin A	94.927	0.488	-0.246	-2.052	N	Y	Y	Y	Y	N	Y	0.534	N	N
7-O-methylglabranin	94.33	-0.101	0.089	-2.185	N	N	Y	Y	Y	N	N	0.092	Y	N
Betulinic acid	100	-1.167	-0.455	-1.048	N	Y	N	N	N	N	N	0.116	N	Y
Dehydrodeguelin	98.492	0.257	-0.278	-1.808	N	Y	Y	Y	Y	N	Y	0.437	N	N
Dehydrorotenone	100	0.432	0.313	-1.843	N	Y	Y	Y	Y	N	Y	0.403	N	N
Flemichapparin B	97.896	0.046	0.201	-1.898	N	Y	Y	Y	Y	N	Y	0.418	Y	N
Lupenone	100	0.04	0.773	-1.694	N	Y	N	N	N	N	N	0.102	N	N
Lupeol	100	-0.088	0.738	-1.43	N	Y	N	N	N	N	N	0.153	N	N
Lupinifolin	90.691	0.442	0	-1.679	N	N	N	Y	Y	N	Y	0.45	N	N
Rotenone	98.921	-0.017	-0.354	-2.717	N	Y	Y	Y	Y	N	Y	0.238	N	N
Stigmasterol	96.39	0.102	0.814	-1.326	N	Y	N	N	N	N	N	0.618	N	N
Tephcalostan	98.391	0.289	-0.681	-1.718	N	Y	Y	Y	Y	N	Y	0.728	Y	N
Tephrinone	93.021	0.137	-0.541	-1.836	N	Y	Y	Y	Y	N	Y	0.22	Y	N
Tephrowatsin C	90.89	0.043	-0.093	-2.227	N	Y	Y	Y	Y	N	Y	0.853	Y	N
Villinol	100	0.362	-0.786	-2.929	N	Y	Y	N	Y	N	Y	0.551	N	N
Villol	69.814	1.052	-1.299	-3.396	N	N	N	N	N	N	N	0.247	N	N
Villosin	100	0.349	0.661	-1.792	N	Y	N	N	N	N	N	1.073	N	Y
Simvastatin	95.313	0.197	-0.37	-2.812	N	Y	N	N	N	N	Y	0.827	N	N
Atovastatin	63.769	-1.44	-1.44	-2.857	Y	Y	N	N	Y	N	N	0.271	N	Y

Ab- Absorption IA- Intestinal Absorption
Db- Distribution VDss – Volume Distribution
Mt- Metabolism BBB- Blood Brain Barrier
Ex- Excretion CNS- Central Nervous System
Tx- Toxicity CYP- Cytochrome P
TC- Total Clearance HT- Hepatotoxicity

CONCLUSION

The 2D structures of the identified compounds (18) were docked with 3 different proteins (1DQ8, 6E7K and 4X96). The docking scores were compared with standard drug namely Atorvastatin and Simvastatin. The potent natural HMG-CoA reductase (HMGR) inhibitors, and activators of LPL and LCAT were chosen based on the binding scores which could be effective molecules in lipid lowering activity. From the docking studies, Tephcalostan, 12a-dehydro-6-hydroxysumatrol, 5,7-di-O-prenylbiochanin A and Villinol bind well at the active site of HMGR and reduce its catalytic activity by conformation modification. Similarly, Tephcalostan, 12a-dehydro-6-hydroxysumatrol, 5,7-di-O-prenylbiochanin A, Lupenone and Lupeol bind and potentiate the enzymatic activity of LPL, and Dehydroeguelin, Tephcalostan, Lupenone and Lupeol stimulates the enzymatic activity of LCAT by binding to its catalytic site. Thus, all the above said natural compounds from Tephrosia villosa and Tephrosia tinctoria could be the drug candidates as antihyperlipidemic agent and that is evidenced by the in silico ADME/T studies and drug likeliness studies..

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

REFERENCES

1. Touiss, I., Harnafi, M., Khatib, S., Bekkouch, O., Ouguerram, K., Amrani, S., and Harnafi, H., (2019) Rosmarinic acid-rich extract from Ocimum basilicum L. decreases hyperlipidemia in high fat diet-induced hyperlipidemic mice and prevents plasma lipid oxidation. *Physiol Pharmacol*, 23, 197-207.
2. Karr, S. (2017) Epidemiology and management of hyperlipidemia. *Am J Manag Care*, 23 (9 Suppl), S139-S148.
3. Reyes-Soffer, G., Ginsberg, H.N., Berglund, L., Duell, P.B., Heffron, S.P., Kamstrup, P.R., Lloyd-Jones, D.M., Marcovina, S.M., Yeang, C., and Koschinsky, M.L., (2022) Lipoprotein(a): A genetically determined, causal, and prevalent risk factor for atherosclerotic cardiovascular disease: a scientific statement from the American heart association. *Arterioscler Thromb Vasc Biol*, 42(1), e48-e60, https://doi.org/10.1161/ATV.0000000000000147.
4. Jang, A.Y., Han, S.H., Sohn, I.S., Oh, P.C., and Koh, K.K. (2020) Lipoprotein(a) and Cardiovascular Diseases - Revisited. *Circ J*, 84(6), 867-874, https://doi.org/10.1253/circj.CJ-20-0051.
5. Gupta, R., Rao, R.S., Misra, A., and Sharma, S.K. (2017) Recent trends in epidemiology of dyslipidemias in India. *Indian Heart J*, 69(3), 382-392, https://doi.org/10.1016/j.ihj.2017.02.020.
6. Yoshida, H., Ito, K., Manita, D., Sato, R., Hiraishi, C., Matsui, S., and Hirowatari, Y., (2021) Clinical Significance of Intermediate-Density Lipoprotein Cholesterol Determination as a Predictor for Coronary Heart Disease Risk in Middle-Aged Men. *Front Cardiovasc Med*, 8, 756057, https://doi.org/10.3389/fcvm.2021.756057.
7. Samuel, V. J., Ramu, M. A., & Vedigounder, M. (2021). Antihyperlipidemic Effect Of Tephrosia Villosa In Acute And Chronic Hyperlipidemia Rat Models. *Journal of Microbiology, Biotechnology and Food Sciences*, 11(2), e4222-e4222.
8. Hoogeveen, R.C., and Ballantyne, C.M., (2021) Residual Cardiovascular Risk at Low LDL: Remnants, Lipoprotein(a), and Inflammation. *Clin Chem*, 67(1), 143-152.
9. Shattat, G.F., (2015) A review article on hyperlipidemia: types, treatments and new drug targets. *Biomed Pharmacol*, 7(1), 399-409.
10. Bouhlali, E.D.T., Hmidani, A., Bourkhis, B., Khouya, T., Harnafi, H., Filali-Zegzouti, Y., and Alem, C., (2020) Effect of Phoenix dactylifera seeds (dates) extract in triton WR-1339 and high fat diet induced hyperlipidaemia in rats: A comparison with simvastatin. *J Ethnopharmacol*., 259, 112961, https://doi.org/ 10.1016/j.jep.2020.112961.
11. Xie, Y., Liu, J., Shi, Y., Wang, B., Wang, X., Wang, W., Sun, M., Xu, X., Jiang, H., Guo, M., He, Y., Ren, C., and Cheng, L., (2021) The combination of Sesamol and Clofibric acid moieties leads to a novel potent hypolipidemic agent with antioxidant, anti-inflammatory and hepatoprotective activity. *Bioorg Med Chem Lett*, 44, 128121, https://doi.org/10.1016/j.bmcl.2021.128121.
12. Sanad, FA., Ahmed, SF., and El-Tantawy, WH., (2020) Antidiabetic and hypolipidemic potentials of Solidago virgaurea extract in alloxan-induced diabetes type 1. *Arch Physiol Biochem*, 1-8. 13813455.2020.1722705.
13. Angiolillo, A., Leccese, D., Palazzo, M., Vizzarri, F., Casamassima, D., Corino, C., and Di Costanzo, A., (2021) Effects of Lippia citriodora leaf extract on lipid and oxidative blood profile of volunteers with hypercholesterolemia: a preliminary study. *Antioxidants (Basel)*, 10(4), 521, https://doi.org/10.3390/antiox10040521.
14. El-Ouady, F., Eddouks, M., (2021) Antihyperglycemic and Antihyperlipidemic Effects of Lippia citriodora in Rats. *Endocr Metab Immune Disord Drug Targets*, 21(4), 711-719.
15. Li, Z., Wu, Y.Y., and Yu, B.X. (2020) Methylophiopogonanone A, an Ophiopogon homoisoflavonoid, alleviates high-fat diet-induced hyperlipidemia: assessment of its potential mechanism. *Braz J Med Biol Res*, 53(3), e9201.
16. Chandramohan, R., and Pari, L., (2021) Antihyperlipidemic effect of tyrosol, a phenolic compound in streptozotocin-induced diabetic rats. *Toxicol Mech Methods*, 31(7), 507-516, https://doi.org/10.108015376516. 2021.1926030.

17. Marahatha, R., Basnet, S., Bhattarai, B.R., Budhathoki, P., Aryal, B., Adhikari, B., Lamichhane, G., Poudel, D.K., and Parajuli, N., (2021) Potential natural inhibitors of xanthine oxidase and HMG-CoA reductase in cholesterol regulation: in silico analysis. *BMC Complement Med Ther*, 21(1), 1.doi.org/10.1186/s12906-020-03162-5.
18. Garg, G., Patil, A., Singh, J., Kaushik, N., Praksah, A., Pal, A., and Chakrabarti, A,. (2018) Pharmacological evaluation of Convolvulus pluricaulis as hypolipidaemic agent in Triton WR-1339-induced hyperlipidaemia in rats. *J Pharm Pharmacol*, 70(11), 1572-1580, https://doi.org/10.1111/jphp.13004.
19. Jasmine, JM., and Vanaja, R., (2013) *In silico* analysis of phytochemical compounds for optimizing the inhibitors of HMG CoA reductase. J App Pharm Sci, 3(9), 43-47, https://doi.org/10.7324/JAPS.2013.3908.
20. Meng, X.Y., Zhang, H.X., Mezei, M., and Cui, M., (2011) Molecular docking: a powerful approach for structure-based drug discovery. *Curr Comput Aided Drug Des*, 7(2), 146-157.doi.org/10.2174/157340911795677602.
21. Siddiqui, H., Yevstigneyev, N., Madani, G., and McCormick, S., (2022) Approaches to Visualising Endocytosis of LDL-Related Lipoproteins. *Biomolecules*, 12(2), 158, https://doi.org/10.3390/biom12020158.
22. Irshad, N., Khan, A.U., Shah, F.A., Nadeem, H., Ashraf, Z., Tipu, M.K., and Li, S., (2021) Antihyperlipidemic effect of selected pyrimidine derivatives mediated through multiple pathways. *Fundam Clin Pharmacol*, 35(6), 1119-1132, https://doi.org/10.1111/fcp.12682.
23. Shaik, AH., Shaik, SR., Daddam, JR., Ali, D., Manoharadas, S., Arafah, MW., and Kodidala, LD. (2021) Maslinic acid and Gallic acid protective efficacy on lipids, lipoproteins and lipid metabolizing enzymes against isoproterenol administered cardiotoxicity: An in vivo and in silico molecular docking evidences. *J King Saud Univ Sci*, 33(1), 101230, https://doi.org/10.1016/j.jksus.2020.101230.
24. Dolgonosov, AM., (2017) The universal relationship between the energy and length of a covalent bond derived from the theory of generalized charges. *Russ J Inorg Chem*, 62(3), 344–350, https://doi.org/10.1134/S0036023617030068.
25. Feingold, K.R. Cholesterol lowering drugs. In: Feingold, K.R., Anawalt, B., Boyce, A., et al., (2021) *Eds., Endotext*. [Internet] MDText.com, Inc.: South Dartmouth (MA), USA, https://www.ncbi.nlm.nih.gov/books/NBK395573/. Accessed April 5, 2022.
26. Goldstein, JL., Brown, MS., (2015) A century of cholesterol and coronaries: from plaques to genes to statins. *Cell*, 161(1), 161-172, https://doi.org/10.1016/j.cell.2015.01.036.
27. Ram, H., Jaipal, N., Charan, J., Kashyap, P., Kumar, S., Tripathi, R., Singh, B.P., Siddaiah, C.N., Hashem, A., Tabassum, B., and Abd Allah, EF., (2020) Phytoconstituents of an ethanolic pod extract of Prosopis cineraria triggers the inhibition of HMG-CoA reductase and the regression of atherosclerotic plaque in hypercholesterolemic rabbits. *Lipids Health Dis*, 19(1), 6, https://doi.org/10.1186/s12944-020-1188-z.
28. Manthei, KA., Yang, SM., Baljinnyam, B., Chang, L., Glukhova, A., Yuan, W., Freeman, LA., Maloney, DJ., Schwendeman, A., Remaley, AT., Jadhav, A., and Tesmer, JJ., (2018) Molecular basis for activation of lecithin:cholesterol acyltransferase by a compound that increases HDL cholesterol. *Elife*, 7e41604, https://doi.org/10.7554/eLife.41604.
29. Chandramohan, R., and Pari, L., (2021) Antihyperlipidemic effect of tyrosol, a phenolic compound in streptozotocin-induced diabetic rats. *Toxicol Mech Methods,* 31(7), 507-516, https://doi.org/10.1080/15376516.2021.1926030.
30. Ramchoun, M., Khouya, T., Harnafi, H., Amrani, S., Alem, C., Benlyas, M., Kasbi, Chadli, F., Nazih, EH., Nguyen, P., and Ouguerram, K., (2020) Effect of Aqueous Extract and Polyphenol Fraction Derived from Thymus atlanticus Leaves on Acute Hyperlipidemia in the Syrian Golden Hamsters. *Evid Based Complement Alternat Med*, 3282596, https://doi.org/10.1155/2020/3282596.
31. Wu, PH., Han, SC., Wu, MH., (2020)Beneficial effects of hydroalcoholic extract from Rosa roxburghii trait fruit on hyperlipidemia in high-fat-fed rats. *Acta Cardiol Sin*, 36(2), 148-159, https://doi.org/10.6515/ACS.202003_36(2).20190709A.
32. Pires, DEV., Blundell, TL., and Ascher, DB., (2015) pkCSM: predicting small-molecule pharmacokinetic and toxicity properties using graph-based signatures. *J Med Chem*, 58(9), 4066–4072, https://doi.org/10.1021/acs.jmedchem.5b00104.
33. Clark, D.E. (2013) In silico prediction of blood–brain barrier permeation. *Drug Discov Today*, 8(20), 927–33, https://doi.org/10.1016/S1359-6446(03)02827-7.
34. Muehlbacher, M., Spitzer, G.M., Liedl, K.R., and Kornhuber, J., (2011) Qualitative prediction of blood–brain barrier permeability on a large and refined dataset. *J Comput Aided Mol Des,* 25(12), 1095–1106, https://doi.org/10.1007/s10822-011-9478-1.
35. Watanabe, R., Ohashi, R., Esaki, T., Kawashima, H., Natsume-Kitatani, Y., Nagao, C., and Mizuguchi., K., (2019) Development of an in silico prediction system of human renal excretion and clearance from chemical structure information incorporating fraction unbound in plasma as a descriptor. *Sci Rep*, 9(1), 18782. https://doi.org/10.1038/s41598-019-55325-1.
36. Samuel, V. J., Rashmi, D. V., & Mahesh, A. R. (2021). Antidiabetic activity of Tephrosia tinctoria in alloxan induced diabetic rats: A preliminary study. *Research Journal of Pharmacy and Technology*, *14*(7), 3727-3732.

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