Research

In Silico Hypolipidemic Activity of Phytoconstitutents Isolated from Coriandrum Sativum Linn Fruits

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Abstract

Aim

Coriandrum sativum Linn is known plant for treatment of lipid lowering effect in Traditional System of Medicine. This study aimed to evaluate molecular interaction of phytoconstituents isolated from C. sativum and targeted protein related to hypolipidemic activity by in silico model.

Material and Methods

Linalool, limonene, stigmasterol and β-sitosterol were isolated from the methanol extract of fruit of C. sativum by column chromatography. Evaluation of hypoglycemic activity through an in silico docking approach with molecular target such as 13-hydroxy-3-methylglutaryl CoA synthase I (HMGCOA) was performed. Molecular docking study was performed with Autodock docking software.

Results

The docking studies of the ligands with target protein showed that this is a good inhibitor, which docks well with -2.02973; -1.57768; -11.7427 and -9.9477 kJ mol\(^{-1}\) Van der Waal energy and -8.8812; -20.7982; -51.3226 and -49.7069 kJ mol\(^{-1}\) as docking energy for linalool, limonene, stigmasterol and β-sitosterol respectively out of which stigmasterol and β-sitosterol has shown activity against the target protein.

Conclusion

Hyperlipidemia treatment is costly medical treatment with lot of side effects. This leads to increasing demand for natural products with hypolipidemic activity with fewer side effects. This work showed that C. sativum extract can be new alternative to existing hypolipidemic treatment.

Keywords: Coriandrum sativum; Hypolipidemia; Molecular Docking; In Silico; Phytoconstituents; 13-hydroxy-3-methylglutaryl CoA synthase I.

Introduction

Hyperlipidemia or hypercholesterolemia is an abnormal elevation in the level of lipids or lipoproteins in our body. Usually level of lipid rises in the blood. Cholesterol is a lipid circulating in our blood. Hypercholesterolemia is a specific case of hyperlipidemia where the level of cholesterol increases in the blood. Cholesterol is insoluble in water; it is transported in the blood plasma with other lipoproteins. Different classifications of lipoproteins include- Very Low Density Lipoprotein (VLDL), Low Density Lipoprotein (LDL), High Density Lipoproteins (HDL), Intermediate Density Lipoprotein (IDL). Cholesterol is carried by all lipoproteins and usually LDL cholesterol is risky known as Bad Cholesterol. Most cholesterol in our body is produced due to internal synthesis in liver. Other sources include dietary sources, genetic influence. Cholesterol has many functions in our body like absorption of fats and hormones like testosterone, estrogen, progesterone, cortisol etc. Cholesterol helps for production of Vitamin D in the presence of sun light. It is essential for providing structural support to cell membranes, serves as antioxidant, and helps in conduction of nerve impulses. An important pathway takes
place in liver called Cholesterol synthesis pathway or Mevalonate pathway. Cholesterol will be the final product of this pathway. Important enzymes involved in this pathway include HMGCoA synthase, Lanoster synthase, and Farnesyl diphosphate synthase [1].

Although increased low density lipoprotein cholesterol (LDL) is thought to be the perfect indicator of atherosclerosis risk, dyslipidemia can also illustrate increased total cholesterol (TC) or triglycerides (TG), or low levels [2]. Altered cholesterol homeostasis contributes to numerous human diseases including atherosclerosis which is the main cause of heart disease, stroke, and death [3-5]. Liver is a key site of de novo cholesterol synthesis. In the absence of dietary cholesterol, increased de novo synthesis in the liver and intestine can meet the cholesterol requirement of all other cells in the body. Under these circumstances, the liver and intestine account for 82 and 11% of total detectable sterol synthetic activity [6]. Inhibition of hepatic cholesterol biosynthesis is potentially an effective approach [7].

Many efforts to figure out the effective treatments for lipid lowering have been increased. Traditional medicinal plants with various active principles and properties have been used from ancient times by physicians and laymen to treat a great variety of human diseases such as coronary heart disease, atherosclerosis etc [8]. There has been increasing demand for the use of plant products with hypolipidemic activity due to low cost, easy availability and lesser side effects. Therefore, plant materials are continuously scrutinized and explored for their effect as hypoglycemic agents. One such plant is Coriandrum sativum which has been used in Traditional System of Indian Medicine for treating hypercholesterolemia.

Coriander [Coriandrum sativum Linn.; Family: Apiaceae] is one of the valuable medicinal and seasoning plant. C. sativum is widely used in traditional medicine to treat anxiety, dizziness, headache, edema, fever, digestive disorders, respiratory diseases, allergies, and burns, astringent, anthelmintic, emollient, stomachic, antibilious, digestive, appetizer, constipating, diuretic, antipyretic, refrigerant, tonic, expectorant, anodyne, antiabetic and dyspepsia. The previous pharmacological studies revealed that it possessed anxiolytic, antidepressant, sedative-hypnotic, anticonvulsant, memory enhancement, improvement of orofacial dyskinesia, neuroprotective, antibacterial, antifungal, anthelmintic, insecticidal, antioxidant, cardiovascular, hypolipidemic, anti-inflammatory, analgesic, anti diabetic, mutagenic, antimutagenic, anticancer, gastrointestinal, deodorizing, dermatological, diuretic, reproductive, hepatoprotective, detoxification and many other pharmacological effects [9-14].

Molecular docking is an important computational tool to predict the plausible interactions between the drug and protein in a non-covalent fashion. An in silico docking procedure have been carried out to examine whether the compound is a good ligand with target 13-hydroxy-3-methylglutaryl CoA synthase I.

The aim of present study was to evaluate the phytoconstituents isolated from methanol extract of C. sativum by docking studies for hypolipidemic activity by in silico technique [2p8u; Crystal structure of human 3-hydroxy-3-methylglutaryl CoA synthase I].

Materials and Methods

Plant material

The Coriandrum sativum fruits were collected from local market in Bangalore, Karnataka, India and it was identified and authenticated by Botanist, Natural Remedies Pvt Ltd., Bangalore. A voucher specimen was deposited in The Oxford College of Pharmacy, Bangalore. The fruits were dried in shade and powdered coarsely, passed through sieve no. 40 and stored in air tight container for further use.

Preparation of fruit extract

Coarsely powdered fruits of C. sativum 200 g was extracted with 75 % methanol [1500 ml] in soxhlet apparatus till the complete exhaustion, filtered. The methanol extract was concentrated by rotary vacuum evaporator and evaporated to dryness.

Chemicals used

All chemicals and solvents used were of analytical grade and procured from SD fine Chemicals Ltd, Mumbai.

Isolation of Linalool, limonene, stigmasterol and β-sitosterol

75% methanol extract was subjected to column chromatography using solvents of increasing polarity as eluent and Silica gel as stationery phase. Fraction from 68-79 from petroleum ether: benzene (98:2) yielded amorphous compound [10 mg]; Fraction from 140-155 from petroleum ether: benzene (95:5) yielded colorless needle shaped compound [10 mg] and Fraction from 180-198 from petroleum ether: benzene (92:8) yielded colorless liquid [50 mg]; Fraction from 105-125 from petroleum ether: benzene (98:2) yielded colorless liquid [50 mg]; Fraction from 105-125 from petroleum ether: benzene (95:5) yielded colorless needle shaped compound [10 mg] and Fraction from 180-198 from petroleum ether: benzene (92:8) yielded amorphous compound [20 mg] which was found to be linalool, limonene, stigmasterol and β-sitosterol and confirmed by Co-TLC and already it was observed in Gas chromatography and by NMR and mass spectrums [15,16].

Sequence retrieval

Authentic structures for Ligands were retrieved from Protein data bank. The three dimensional structure of target protein was downloaded from PDB (www.rcsb.org/pdb) structural database. This file was then opened in SPDB viewer edited by removing the heteroatoms, adding C terminal oxygen. The active pockets on target protein molecule were found out using CASTp server. The ligands were drawn using ChemDraw Ultra 6.0 and assigned with proper 2D orientation (ChemOffice package). 3D coordinates were prepared using PRODRG server.
Ligand binding site prediction

Ligand binding sites were calculated using Q site finder http://www.modelling.leeds.ac.uk/qsitefinder/, surface topology and the pocket information were also analyzed by the cast P server http://sts-fw.bioengr.uic.edu/castp/calculation.php. Pocket detection and occupancy of the protein was set up using Q-Site Finder. The solvent available surface area (SASA) was found by the software server GETAREA http://curie.utmb.edu/getarea.html. The atomic Solvent Accessible Surface Area (SASA) enclosed by each cleft was calculated by utilizing radius of water probe 1.4 Å and the area/energy per residue was also designed. Dielectric constant was set to a value of 80.0, and Poisson-Boltzmann method of computation for 20 cycles was used for calculating the electrostatic potential in SWISS-PDB viewer. All the ligand binding residues were amongst hotspots as predicted by Meta-PPISP. Furthermore, PIC was made to use to calculate the nature of interaction occurring in the ligand binding residues.

Docking studies

Autodock V3.0 was used to perform Automated Molecular Docking in AMD Athlon (TM)2×2 215 at 2.70 GHz, with 1.75 GB of RAM. AutoDock 3.0 was compiled and run under Microsoft Windows XP service pack 3. For docking, grid map is required in AutoDock, the size of the grid box was set at 102, 126 and 118 Å (R,G, and B), Comparative availability of 3D structures was checked in NCBI Entrez, along with PDB and SWISS-PROT databases [17].

Assessment of protein-ligand interaction

Hydrogen bond interactions were calculated by using Discovery studio (http://accelrys.com/products/discovery-studio) and ligand map was generated using MOLEGRID (http://molegro-molecular-viewer.software.informer.com/2.5/).

Results and Discussion

Linalool, limonene, stigmasterol and β-sitosterol were isolated from 75% methanol extract of C. sativum fruits. The structures are given in the Figure 1A-1D.

Linalool was obtained as colorless liquid, bp:198°C [lit bp: 198-199°C], refractive index: 1.460 [lit refractive index: 1.463]. Molecular formula: C_{10}H_{18}O and molecular weight: 154. It was confirmed to be Linalool by Co-TLC.

Limonene was obtained as colorless liquid, bp:175°C [lit bp: 176°C], refractive index: 1.470 [lit refractive index: 1.4727]. Molecular formula: C_{10}H_{16}. It was confirmed to be Limonene by Co-TLC.

Compound 3 was crystallized as colorless needles crystals. Positive test for Liebermann Burchardt test indicated the presence of tetracyclic triterpenoid compound. The molecular formula was established as C_{18}H_{28}O_{5} by HR-FAB-MS which showed molecular ion peak at m/z 574.4231 (calcd. for C_{18}H_{28}O_{5}, 574.4233), MP 289 – 290°C. The ^1H-NMR as well as ^13C-NMR data were found to be identical with the spectrum of those already reported earlier for stigmasterol [18]. It was further confirmed by TLC and CO-TLC method with the reference standard of stigmasterol.

Compound 4 was isolated as white amorphous powder, m.p.:139-142°C. Positive test for Liebermann Burchardt test indicated the presence of tetracyclic triterpenoid compound. Its IR spectrum exhibited characteristic bands at 3288 cm^{-1} for hydroxyl group. The ^1H-NMR as well as ^13C-NMR data were found to be identical with the spectrum of those already reported earlier for β-sitosterol [19]. It was further confirmed by TLC and CO-TLC method with the reference standard of β-sitosterol.
This isolations are already reported in paper published [16].

**In silico molecular docking studies**

In order to investigate the binding capacity of Linalool, limonene, stigmasterol and β-sitosterol in *C. sativum* on proteins related to lipid lowering effect in humans, we docked the compound to the target protein. Results showed that dock of the molecule with -2.02973; -1.57768; -11.7427 and -9.4477 kJ mol⁻¹ Van der Waal energy and -8.8812; -20.7982; -51.3226 and -49.7069 kJ mol⁻¹ as docking energy for linalool, limonene, stigmasterol and β-sitosterol respectively (Table 1). High binding affinity of the ligand to the receptor was explained clearly by interaction analysis in Figure 2 to Figure 5.

### Table 1: Molecular docking results of linalool, limonene, stigmasterol and β-sitosterol with 2p8u

<table>
<thead>
<tr>
<th>Molecule</th>
<th>Initial potential energy</th>
<th>Potential Energy</th>
<th>Initial RMS gradient</th>
<th>RMS gradient</th>
<th>Van der Waals energy</th>
<th>CDocker Energy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Linalool</td>
<td>50.9046</td>
<td>20.0971</td>
<td>12.1566</td>
<td>0.09716</td>
<td>-2.02973</td>
<td>-8.8812</td>
</tr>
<tr>
<td>Limonene</td>
<td>59.4386</td>
<td>34.741</td>
<td>16.4867</td>
<td>0.09321</td>
<td>-1.57768</td>
<td>-20.7982</td>
</tr>
<tr>
<td>Stigmasterol</td>
<td>194.408</td>
<td>73.5711</td>
<td>26.6645</td>
<td>0.38468</td>
<td>-11.7427</td>
<td>-51.3226</td>
</tr>
<tr>
<td>β-sitosterol</td>
<td>311.053</td>
<td>80.1545</td>
<td>62.2544</td>
<td>0.21535</td>
<td>-9.9477</td>
<td>-49.7069</td>
</tr>
</tbody>
</table>

**Figure 2:** Linalool interactions with the active site amino acids of the 2p8u protein

**Figure 3:** Limonene interactions with the active site amino acids of the 2p8u protein

**Figure 5:** β-Sitosterol interactions with the active site amino acids of the 2p8u protein
HMG-CoA synthase (PDB ID: 2P8U) catalyzes the condensation of acetoacetyl-CoA and acetyl-CoA to form HMG-CoA plus free CoA. It is the second reaction in the mevalonate-dependent isoprenoid biosynthesis pathway. HMG-CoA is an intermediate in both cholesterol synthesis and ketogenesis. This reaction is over-activated in patients with diabetes mellitus type I if left untreated, due to prolonged insulin deficiency and the exhaustion of substrates for gluconeogenesis and the TCA cycle, notably oxaloacetate. This results in shunting of excess acetyl-CoA into the ketone synthesis pathway via HMG-CoA, leading to the development of diabetic ketoacidosis [20].

HMG-CoA synthase activity is located in two different compartments, namely the cytosol and the mitochondria [21]. The mitochondrial HMGCoA synthase is the rate-limiting enzyme of the ketogenic pathway, in studies of acetoacetate production in sonicated liver particles was first proposed [22]. HMG-CoA synthase (2P8U) which produce HMG-CoA is the starting point of the isoprenoid pathway where the cholesterol is the main end product. This enzyme is a potential drug target for regulating serum cholesterol level [23].

HMG-CoA synthase contains an important catalytic cysteine residue that acts as a nucleophile in the first step of the reaction: the acetylation of the enzyme by acetyl-CoA (its first substrate) to produce an acetyl-enzyme thioester, releasing the reduced coenzyme A. The subsequent nucleophilic attack on acetoacetyl-CoA (its second substrate) leads to the formation of HMG-CoA [24].

In our study, docking of novel compound with 2P8U showed Van der Waal and other interactions with as follows for 4 compounds.


Conventional Hydrogen bond: SER A: 221.


**Stigmasterol**: Van der Waal interaction with GLY A:218; LYS A:46; GLY A:45; ALA A:168; GLY A:50; ILE A:222;ARG A:313; LYS A:269; LYS A:273; THR A:171.


**β-sitosterol**: Van der Waal interaction with SER A: 221; ARD A: 313; ASN A: 167; LYS A: 46.


4 isolated compounds have shown good inhibitory activity for this target protein which is evident by the docking energy and van der Waal interaction out of which stigmasterol and β-sitosterol has the best activity. Hence this could be potential leads for hypolipidemic activity.

Linalool isolated from the methanol extract has shown in silico activity against the target proteins, glutamine: fructose-6-phosphate amidotransferase and 4 compound isolated have shown activity against 11β-hydroxysteroid dehydrogenase type I [25,16].

**Conclusion**

All the compounds were found to interact the target protein which can be related to DM with -2.02973; -1.57768; -11.7427 and -9.9477 kJ mol⁻¹ Van der Waal energy and -8.8812; -20.7982; -51.3226 and -49.7069 kJ mol⁻¹ as docking energy for linalool, limonene, stigmasterol and β-sitosterol respectively out of which stigmasterol and β-sitosterol has shown activity against the target protein. Docking studies of the compounds with target protein showed that stigmasterol and β-sitosterol is promising candidate which docks well with the target related to cholesterol lowering effect which could be considered for developing into hypolipidemic drug. In silico activity might be one of the potent ways of finding a possible molecule for such activity. In fact in plant extract, it is the holistic approach has always shown better activity when compared to individual compounds.

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**References**


