Research Article

In Silico Antidiabetic Activity of Limonene Isolated from *Coriandrum sativum* Linn Fruit

M Padmaa Paarakh*

Department of Pharmacognosy, The Oxford College of Pharmacy, Bangalore, Karnataka, India

***Corresponding Author:** M Padmaa Paarakh, Principal, Department of Pharmacognosy, The Oxford College of Pharmacy, Bangalore, Karnataka, India.*

Abstract

Background and Aim: Diabetes mellitus [DM] is a metabolic disorder characterized by disturbances in carbohydrate, protein and lipid metabolism and by complications like micro vascular (retinopathy, neuropathy and nephropathy) and macro vascular (heart attack, stroke and peripheral vascular disease) complications. *Coriandrum sativum* Linn has been claimed to possess antidiabetic properties in Traditional System of Medicine. This study aimed to evaluate molecular interaction of limonene in *C. sativum* and targeted protein related to Type 2 DM.

Material and Methods: Isolation of Limonene from the methanol extract of fruit of *C. sativum* was done by column chromatography. Evaluation of hypoglycemic activity through an *in silico* docking approach with molecular target such as glutamine: fructose- 6-phosphate amidotransferase was performed. Molecular docking study was performed with Autodock docking software.

Results: The docking studies of the ligand limonene with target protein showed that this is a good inhibitor, which docks well related to diabetes mellitus with -1.57768 kJ mol^{-1} Van der waal energy and -17.6701 kJ mol^{-1} as docking energy. Hence limonene can be considered for developing into a potent anti-diabetic drug.

Conclusion: Management of diabetes is challenge to the medical treatment. This leads to increasing demand for natural products with antidiabetic activity with fewer side effects. This work establishes *C. sativum* extract as a potential inhibitor for diabetes. Thus enabling a possibility of this plant extract as a new alternative to existing diabetic treatment.

Keywords: Coriandrum sativum*; Diabetes Mellitus; Molecular Docking; *In Silico;* Limonene*

Introduction

Diabetes mellitus is a metabolic disorder characterized by disturbances in carbohydrate, protein and lipid metabolism and by complications like micro vascular (retinopathy, neuropathy and nephropathy) and macro vascular (heart attack, stroke and peripheral vascular disease) complications [1]. A world-wide survey has reported that diabetes mellitus affects nearly 10% of the population. It has been predicted that the prevalence of diabetes in adults will increase from 135 million in 1995 to 350 million in 2030 as given by International Diabetes Federation [2,3]. Currently available synthetic antidiabetic agents produce serious side effects like hypoglycemic coma and hepatorenal disturbances [4,5]. Patients are therefore using herbal medicines which have fewer side effects and have the potential to impart therapeutic effect in complicated disorders like diabetes and its complication [6].

Plants are well known in traditional herbal medicine for their hypoglycemic activities and available literature indicate that there are more than 800 plant species showing hypoglycemic activity. There has been increasing demand for the use of plant products with antidiabetic 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 diabetes.

Coriander [*Coriandrum sativum* Linn.] an annual of the Apiaceae family 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 [7,8]. The fruits are used as astringent, anthelmintic, emollient, stomachic, antibilious, digestive, appetizer, constipating, diuretic, antipyretic, refrigerant, tonic, expectorant, anodyne, antidiabetic and dyspepsia. The phytochemical screening of *Coriandrum sativum* showed that it contained essential oil, tannins, terpenoids, reducing sugars, alkaloids, phenolics, flavonoids, fatty acids, sterols, glycoside, wide range of minerals, trace elements and vitamins. 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, antidiabetic, mutagenic, antimutagenic, anticancer, gastrointestinal, deodorizing, dermatological, diuretic, reproductive, hepatoprotective, detoxification and many other pharmacological effects [9-14].

Citation: M Padmaa Paarakh*. "In Silico* Antidiabetic Activity of Limonene Isolated from *Coriandrum sativum* Linn Fruit". *Acta Scientific Pharmaceutical Sciences* 2.1 (2018): 02-06.

Silver nanoparticles were synthesized using methanol and aqueous extract of fruit of *C. sativum* and its antioxidant activity were reported [15]. We have reported better activity with 75% methanol extract of fruit of *C. sativum* and HPTLC data also showed that 75% methanol extract has more number of phytoconstituents than all other extract [10]. The 75% methanol extract showed significant decrease in blood glucose level at a dose of 100 mg/kg and 200 mg/kg. It also decreased the lipid parameters such as total cholesterol, total triglycerides, total bilirubin, SGOT, SGPT and ALP when compared with diabetic control. 75% methanol extract at 100 mg/kg was better in controlling diabetes when compared to 200 mg/kg bw suggesting that *C. sativum* possess antidiabetic activity [16,17]. Linalool isolated from *C. sativum* have shown *in silico* antidiabetic activity [18].

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 diabetic target Glutamine: fructose-6-phosphate amidotransferase. Glutamine: fructose-6-phosphate amidotransferase (GFAT) is a rate-limiting enzyme in the hexoamine biosynthetic pathway and plays an important role in type 2 diabetes. The enhanced activity of human GFAT has been implicated in insulin resistance in cellular and animal models. Thus, human GFAT is recognized as an interesting potential target for type 2 diabetes complications in medicinal chemistry. Glutamine-fructose-6-phosphate amidotransferase (GFAT or GFPT) is the first and rate-limiting enzyme of the hexosamine pathway. GFAT controls the flux of glucose into the hexosamine pathway and catalyzes the formation of glucosamine 6-phosphate. The majority of glucose will enter the glycolysis pathway, with a small percentage entering the hexosamine pathway. GFPT or GFAT regulate the hexosamine pathway products. Therefore, this enzyme involved in a therapeutic target against Type 2 DM [19].

Aim of the Study

The aim of present study was to isolate Limonene from methanol extract of *C. sativum*. To the best of our knowledge, this is the first report on docking studies of the compound Limonene isolated from *C. sativum* for antidiabetic activity.

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* 200g 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 Limonene

75% methanol extract was subjected to column chromatography using solvents of increasing polarity as eluent and Silica gel

as stationery phase. Fraction from 105 - 125 from petroleum ether: Benzene (98:2) yielded colorless liquid [50 mg; 0.25%] which was found to be limonene and confirmed by Co-TLC and already it was observed in Gas chromatography [20].

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. Clefts were tarnished in the protein-surface using Q-SiteFinder. The solvent available surface area (SASA) was found by the software server GE-TAREA 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 A^0 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.
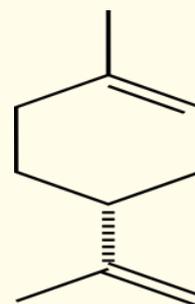
Docking studies

Autodock V3.0 was used to perform Automated Molecular Docking in AMD Athlon (TM) 2x2 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 SWISSPROT databases [21].

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 MOLEGRO (http://molegro-molecular-viewer.software.informer.com/2.5/).

Results and Discussion

Limonene was isolated from 75% methanol extract of *C. sativum* fruits. The structure is given in the figure 1. It was obtained as colorless liquid, bp: 175^{0}C [lit bp: 176^{0}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.


Figure 1: The structure of Limonene [Synonym: 1-Methyl-4-(1-methylethenyl)cyclohexene].

In silico molecular docking studies

In order to investigate the binding capacity of Limonene in *C. sativum* on proteins related to diabetes in humans, we docked the compound to the target protein. Results showed that dock of the molecule with -1.57768 kJ mol^{-1} Van der waal energy and -17.6701 kJ mol^{-1} as docking energy (Table 1). High binding affinity of the ligand to the receptor was explained clearly by interaction analysis in figure 2-4.
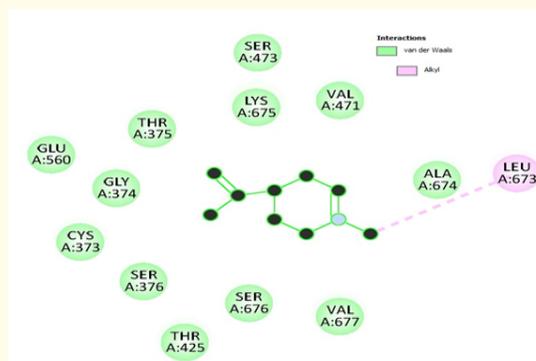

Figure 2: Limonene interactions with the active site amino acids of the 2zj3 protein.
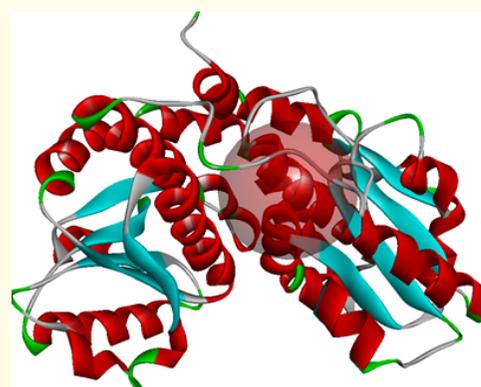
Figure 3: Secondary structure and binding site of the 2zj3 protein.

Molecule	Initial potential energy	Potential Energy	Initial RMS gradient	RMS gradient	Van der Waals energy	CDocker Energy
Limonene	59.4336	34.741	16.4867	0.09321	-1.57768	-17.6701

Table 1: Molecular docking results of limonene with 2zj3.
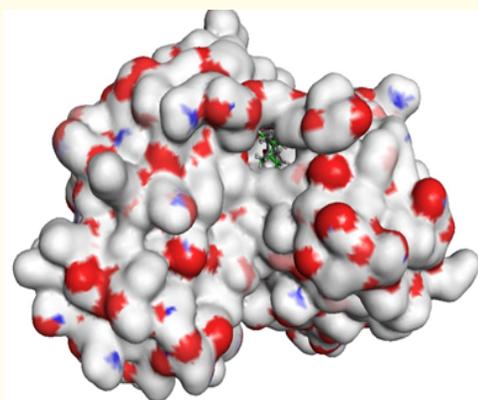
Figure 4: Surface view and docked ligand inside the 2zj3 protein.

Glutamine: fructose-6-phosphate amidotransferase (GFAT) is the key enzyme in the hexosamine biosynthetic pathway and plays an important role in type 2 diabetes [22]. In our study, docking of novel compound with 2ZJ3 showed van der Waal interaction with GLU A:568, THR A:375, SER A:473, LYS A:675, VAL A:471, GLY A:374, CYS A:373, SER A:376, CYS A:373, SER A:376, THR A:425, SER A:676, VAL A:677 and ALA A:674.

GFAT plays a key role in hexosamine biosynthetic pathway, which is involved in glucose-induced insulin resistance and the induction of the synthesis of growth factor. The hexosamine pathway is the sensor of the glycoglysis pathway, in the reaction of fructose-6-phosphate (F6P) with the NH2 donor amino acid, a glutamine which is converted to glucosamine-6-phosphate (GlcN6P) by the glutamine fructose-6-phosphate amidotransferase (GFA) enzyme. Subsequent reactions result in the formation of uridine diphosphate N-acteylglucosamine (UDP-GlcNAc) which is utilized as a substrate for N- and O-linked glycosylation. It is intracellular protein O-glyco-sylation mediated by O-linked GlcNAc transferase (OGT) which has been postulated to contribute to glucose toxicity by altering gene expression. The synthesis of glucosamine begins with the transport of glucose into the cell and its conversion to glucose-6-phosphate by hexokinase and then isomerization to fructose-6-phosphate by the enzyme phosphofructose kinase I of the glycolysis pathway. Then fructose-6-phosphate can go through the normal metabolic pathway as previously described in glucose metabolism. Due to the hexosamine pathway, 2 - 5% of fructose-6-phosphate formed in glycolysis is converted to glucosamine. Fructose-6-phosphate is isomerized and aminated by glutamine and GFAT (L-glutamine, D-fructose-6-phosphate amidotransferase) to yield glucosamine-6-phosphate. First, acetyl-coenzyme (CoA) from either glucose metabolism or fatty acid oxidation transfers its acetyl group to glucosamine-6-phosphate to produce N-acetylglucosamine-6-phosphate. Then, a uridine nucleotide (UDP) is added to the glucosamine yielding uridine 50-diphospho-N-acetylglucosamine (UDP-GlcNAc). Overall the production of UDP-GlcNAc requires glucose, glutamine, acetyl-CoA, uridine, and ATP. Studying the interaction of GFAT with UDP-GlcNAc and its 3D structure will provide more chances to develop a new strategy for treatment of type 2 diabetes. The majority of glucose will enter the glycolysis pathway, with a small percentage entering the hexosamine pathway. Hexosamine biosynthesis is known to contribute to insulin resistance and the induction of the synthesis of growth factor. GFPT or GFAT regulate the hexosamine pathway products. Therefore, this is involved in a therapeutic target against type 2 diabetes [19].

Conclusion

Renewed interest on the use of herbal products is gaining importance. Globally, many herbal products are being used as food supplements for prophylaxis of common ailments such as diabetes, hypertension etc.

The 75% methanol extract showed significant decrease in blood glucose level at a dose of 100 mg/kg and 200 mg/kg. It also decreased the lipid parameters such as total cholesterol, total triglycerides, total bilirubin, SGOT, SGPT and ALP when compared with diabetic control. 75% methanol extract at 100 mg/kg was better in controlling diabetes when compared to 200 mg/kg bw suggesting that *C. sativum* possess antidiabetic activity. This was further confirmed by isolating Limonene from the methanol extract. This compound was found to interact the target protein which can be related to DM -1.57768 kJ mol-1 Van der waal energy and -17.6701 kJ mol-1 as docking energy. Docking studies of the limonene with target protein showed that this is a promising candidate which docks well with the target related to diabetes mellitus. Thus, Limonene can be considered for developing into a potent antidiabetic drug.

Acknowledgement

Conflict of Interest

None to be declared.

Bibliography

1. Umar A., *et al*. "Anti-hyperglycemic activity of the leaves of Tetracera scandens Linn. Merr. (Dilleniaceae) in alloxan induced diabetic rats". *Journal of Ethnopharmacology* 131.1 (2010): 140-145.

2. Menaka CT., *et al*. "Prevention of high fat diet induced insulin resistance in mice by Sida rhomboidea ROXB. Extract". *Journal of Health Sciences* 56.1 (2010): 92-98.

3. Amos AF., *et al*. "The rising global burden of diabetes and its complications: estimates and projections by 2010". *Diabetic Medicine* 14.5 (1997): S1-S85.

4. Sunil C., *et al*. "Effect of ethanolic extract of Pisonia alba Span. leaves on blood glucose levels and histological changes in tissues of alloxan induced diabetic rats". *International Journal of Applied Research in Natural Products* 2.2 (2009): 4-11.

5. Kyriacou A and Ahmed AB. "Exenatide use in the management of type 2 diabetes mellitus". *Pharmaceuticals* 3.8 (2010): 2554-2567.

6. Pandita R., *et al*. "Antidiabetic effect of Ficus religiosa extract in streptozotocin induced diabetic rats". *Journal of Ethnopharmacology* 128.2 (2010): 462-466.

7. Bently R and Trimen H. "Medicinal Plants". Omsons Publications, New Delhi, India, 2 (2002): 729.

8. Khare PC. "Indian Medicinal Plants". Springer Science Publishers, NY, USA (2007): 174.

9. Padmaa MP. "Coriandrum sativum Linn: A review". *Pharmacologyonline News Letters* 3 (2009): 561-573.

10. Padmaa MP., *et al*. "HPTLC finger profile of Coriandrum sativum Linn". *Research Journal of Pharmaceutical, Biological and Chemical Sciences* 6.1 (2015): 1407-1415.

11. Sangeeta PB., *et al*. "Dose dependent effect of Coriandrum sativum seeds on thermal pain stimuli". *Journal of Phytopharmacology* 3.4 (2014): 254-258.

12. Kamempor SF., *et al*. "The analgesic effect of different extracts of aerial parts of Coriandrum sativum in mice". *International Journal of Biomedical Science* 11.1 (2015): 23-28.

13. Padmaa MP. "Comparison of in vitro antioxidant activity of Coriandrum sativum fruit by soxhlation and maceration extraction technique". *European Journal of Biomedical and Pharmaceutical Sciences* 1 (2014): 230-240.

14. Padmaa MP., *et al*. "Analgesic activity of methanol extract of Coriandrum sativum Linn fruit". *World Journal of Pharmacy and Pharmaceutical Sciences* 5.10 (2016): 1456-1464.

15. Padmaa MP. "Green synthesis of silver nanoparticles using fruits of Coriandrum sativum Linn and its antioxidant activity". *Journal of Natural Products and Resources* 1.1 (2015): 19-22.

16. Padmaa MP. "Antidiabetic activity of methanol extract of Coriandrum sativum Linn fruit". *European Journal of Biomedical and Pharmaceutical Sciences* 4.2 (2017): 355-359.

17. Padmaa MP. "Histopathology studies of methanol extract of Coriandrum sativum Linn fruit on streptozotocine induced diabetic rat". *European Journal of Biomedical and Pharmaceutical Sciences* 4.2 (2017): 385-389.

18. Padmaa MP. "In Silico antidiabetic activity of linalool isolated from Coriandrum sativum Linn fruit". *International Journal of cancer and cellular Biology Research* 2.2 (2017): 29-33.

19. Chou K C. "Molecular therapeutic target for type-2 diabetes". *Journal of Proteome Research* 3.6 (2004): 1284-1286.

20. Padmaa MP. "Analysis of oil composition of the methanol extract of Coriandrum sativum fruit by soxhlation technique". *International Journal of Current Pharmaceutical Research* 6.4 (2014): 53-54.

21. Lipinski CA., *et al*. "Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings". Advanced Drug Delivery Reviews 23.1-3 (1997): 23-25.

22. Nakaishi Y., *et al*. "Structural analysis of human glutamine: fructose-6-phosphate amidotransferase, a key regulator in type 2 diabetes". *FEBS Letters* 583.1 (2009): 163-167.