In-Silico Analysis Of Hydroxy Citric Acid From *Pithecellobium Dulce Benth* As A Treatment For Obesity-Related Diabetes Mellitus

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Abstract:

Diabetes mellitus is an endocrine system metabolic disorder It occurs when the body does not make enough insulin or does not utilize it efficiently. The P*ithecellobium dulce* Benth commonly known as schezwan pepper belongs to the Rutaceae family used as a traditional medicine for several years, and also used for culinary purposes. High anti-oxidant property of the plant makes it as a favourable choice in the research field. In the present study, we have extracted and characterized Hydroxy Citric Acid from *Pithecellobium dulce* Benth and performed In-silico evaluation for the finding its activity against Obesity-Related Diabetes Mellitus Extraction of Hydroxy Citric Acid from the *Pithecellobium dulce* Benth seeds and were subjected to in silico studies to identify the activity of the compound for Obesity-Related Diabetes Mellitus. Molecular docking studies were performed against Phosphorylated insulin receptor tyrosine kinase in complex with peptide substrate and ATP analog (PDB ID: 1RI3) From the binding interactions, binding energies and ADME predictions the compound showed good predicted values and were characterised by using FTIR & HPLC.

Keywords: AutoDock, Hydroxy Citric Acid, Tyrosine kinase, pithecellobium dulce Benth

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Introduction:

Natural products are becoming a more compelli ng candidate in drug discovery. According to a detailed analysis of new medicines, natural products and its derivatives approved by Food and Drug Administration accounted for 34% in this year by between 1981 and 2010, including statins, tubulin-binding anticancer drugs, and immunosuppressants. This contribution of natural products seems impressive, especially against the antidiabetic property. Drug discovery industry recently started focusing on natural products due their vast nature of activity along with less toxicity. (1)

Diabetes mellitus is an endocrine system metabolic disorder. Diabetes patients cannot produce or use insulin adequately, resulting in excessive blood glucose levels. Type 2 diabetes, also known as non–insulin–dependent diabetes mellitus, is the most prevalent type of diabetes, accounting for 90 to 95 percent of cases. It occurs when the body does not make enough insulin or does not utilise it efficiently. Because of an increase in the number of senior individuals, as well as a higher incidence of obesity and a sedentary lifestyle, Type 2 diabetes is increasing in large number. Many of these oral antidiabetic agents have a number of serious adverse effects; thus, managing diabetes without any side effects is still a challenge.(2)

The Pithecellobium dulce Bench belongs to the Leguminosae family used as a traditional medicine for several years, and also used for

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culinary purposes. High anti-oxidant property of the plant makes it as a favourable choice in the research field. Hydroxy Citric Acid (HCA) extracted from the seeds of *Pithecellobium dulce* Benth are used as antiobesity agent to reduce the cholesterol level by inhibition of mitochondrial citratelyase causing lower acetyl coenzyme A and reduced fatty acid synthesis and also revealed that the plant extract has been used for antidiabetic activity. (3)

In the present study, we have extracted and characterized Hydroxy Citric Acid from *pithecellobium dulce* Benth and performed *In-silico* evaluation for the finding its activity against Obesity-Related Diabetes Mellitus.

MATERIALS AND METHODS:

Collection of plant and Software requirement:

The chemicals of analytical grade are procured from Merck-Sigma Aldrich and they are used for the study. IR was performed by SHIMADZU IRTracer-100 FTIR spectrophotometer. Software's and online tools like ChemDraw Professional 16.0, Open Babel 3.1.1, Avogadro 1.2.0, Autodock 4.2.6 software, Pro Tox II, AdmetSAR-2.0, Molinspiration, Discovery studio visualizer 3.0 were used for identifying *in-silico* activity. The seeds of pithecellobium dulce. were collected from iStore Direct Trading LLP, Mumbai.

Extraction and characterization of HCA:

150 g of dry *Z. rhetsa* was taken and stirred with 750mL of distilled water using mechanical stirrer for 30 min. The solution was filtered using Whatman filter paper No.1 and the filtrate was collected. The filtrate was mixed with1 N calcium hydroxide solution and stirred using magnetic stirrer until the pH reached 7.0. Calcium salt of HCA precipitated out and collected by subsequent filtration and dried in hot air oven to get salt of Calcium hydroxy citrate and the quantity of HCA extracted was noted. The extracted HCA from were subjected to elemental analysis using FTIR for characterization studies.(4)

Molecular Docking*:*

Molecular docking was performed using Autodock 4.2.6 software. Two dimensional structures of Hydroxy Citric acid were generated using ChemDraw Professional 16.0, and Avogadro 1.2.0 was used for energy minimization. X-ray crystal structure of Phosphorylated insulin receptor tyrosine kinase in complex with peptide substrate and ATP analog (PDB ID: 1RI3) were taken from the protein data bank (RCSB). The best protein-ligand complex was determined by docking energy, score and active site of interactions were analysed using discovery studio visualizer 3.0.(5-7)

Toxicity Prediction:

The Pro tox II an open-source web server, is used to calculate in silico models for toxicity prediction which determines the features of mutagenicity, carcinogenicity, irritability, and reproductive impact.(8)

ADME Prediction:

An online admetSAR-2.0 webserver was used to predict adsorption, distribution, metabolism and elimination. AMES toxicity, carcinogenicity, acute oral toxicity, and human gene inhibition was evaluated.(9-10)

Physiochemical Property:

Physiochemical property was calculated by Molinspiration webserver according to the Lipinski's rule of five (RO5), all chemical structures should follow the rule and should have good values when compared with standard values of molecular weight (<5000 g/mol), HBA (<10), HBD (<5) and logP (<5) values. The Lipinski rule violation causes compounds with less absorption. Hydrophobicity, electronic distribution, hydrogen bonding properties, molecule size, and flexibility are all key molecular factors that go into the drug score. (11-15)

RESULTS AND DISCUSSION:

Molecular docking*:*

Molecular Docking was performed using Autodock to determine the binding energy against insulin receptor tyrosine kinase target. The docking of the ligand molecule indicates that all inhibitor compounds in the active pockets are bonded to one or more amino acids. The theoretical binding energy of HCA was found to be of **-4.44 kcal/mol** and showed good binding energies when compared to the standard Metformin **-4.63 kcal/mol.** 2D and 3D

View of the Binding conformation were shown in Figure.1

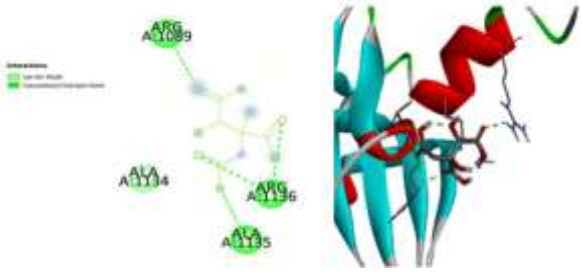

Figure.1 2D and 3D View of the Binding conformation of hydroxycitric acid

ADME Properties:

The compound was subjected for ADME prediction using AdmetSAR and the result were tabulated in Table 1. Predicted properties of HCA were within the range.

Table 1: ADMET Parameters by AdmetSAR

Compound	HIA	BBB	CYP inhibition/substrate	LD50 in rat
Hydroxy Citric Acid	0.7518	0.8550	Non-Substrate /inhibitor	1.7748

Toxicity Prediction:

When working with in silico toxicity models, overall toxic phenomena, such as carcinogenicity, mutagenicity and other models that contribute to toxicity manifestations are identified by using Pro tox II. It was found to be non-toxic and have good drug-like properties and the results were summarized in the Table 2.

Table 2: Toxicity Profile by Osiris property explorer.

Compounds	Mutagenicity	Carcinogenicity	Irritant	Reproductive Effect	Drug-Likeness
Hydroxy Citric Acid	Non-toxic	Non-toxic	Non-toxic	Non-toxic	0.97

Physiochemical Property:

Our findings revealed that HCA had high drug score values, indicating that they had good drug-like behaviour and may be used as drug candidates. The values predicted is given in the Table 3.

Table 3: Physiochemical Property by Molinspiration.

Compounds	Log P	Molecular Weight	No. of Rotatable bonds	No. of Hydrogen Donors	No. of Hydrogen Acceptors	Violations
Hydroxy Citric Acid	2.90	208.12	5	5	5	0

Chemistry:

HCA (1,2-dihydroxypropane-1,2,3-tricarboxyli-cacid) has two asymmetric canters, hence two pairs of distereoisomers (+)-HCA, (-)-HCA are possible. Fig. The isolated active compound was confirmed using Phytochemical analysis and chemical tests. The HPTLC and FTIR spectrum of HCA showed similar pattern at wave number between 1600 and 1070 cm-1 that confirmed the presence of HCA.(10) (11)

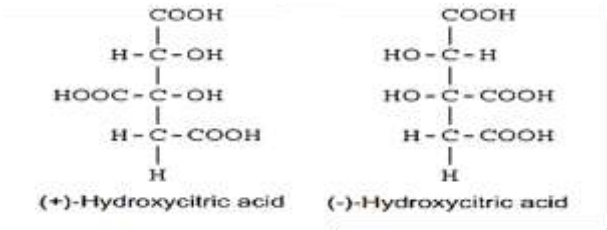

Figure.2 Structures of hydroxycitric acid isomers

HCA (1,2-dihydroxypropane-1,2,3-tricarboxylic acid) has two asymmetric centers; hence two pairs of distereoisomers or four different isomers are possible (Fig. 1). All four isomers, ())-HCA, (+)-HCA, ())-allo-HCA and (+)-allo-HC

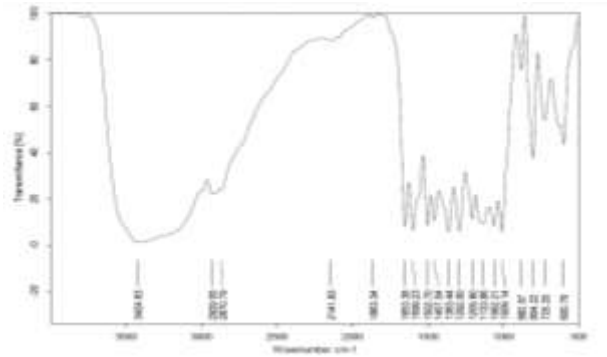

Figure.3 FTIR spectrum of HCA extracted from *Zanthoxylum rhetsa*

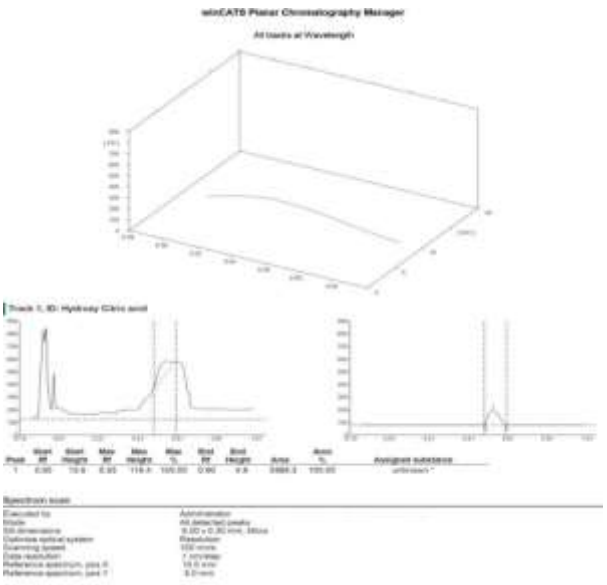

Figure.4 HPLC chromatogram of HCA extracted from *Zanthoxylum rhetsa*

Conclusion:

Medicinal plants constitute a rich source of bioactive chemicals that are largely free from adverse effects and have excellent pharma-cological actions. Hydroxy citric acid from *pithecellobium dulce* was extracted and in silico studies like molecular docking, physiochemical property and Admet prediction showed good predicted values and characterised by using FTIR & HPLC. The findings of this study can be utilized in further research to develop better anti-diabetic drugs with novel targets.

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