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PHYTOCHEMICAL ANALYSIS AND MOLECULAR DOCKING STUDIES OF ISOLATED COMPOUND FROM *ZANTHOXYLUM RHETSA* LINN AS A TREATMENT FOR OBESITY-RELATED DIABETES MELLITUS

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ABSTRACT

Objective

Isolation of hydroxy citric acid from the *Zanthoxylum rhetsa* Linn seeds schezwan pepper and to evaluate this Obesity-related Diabetes mellitus

Methods

The isolated compound of hydroxy citric acid obtained from *Zanthoxylum rhetsa* Linn seed by using the standard method and confirmed by using chemical test and spectral studies, this isolated compound was used to further studies like evaluation of glucose uptake assay by using cell lines like 3T3-L1 cell lines Molecular docking studied were performed on Human Aldol Reductase (PDB 3RX2), Human Mineralocorticoid receptor (PDB 3VHU) receptors using Auto dock 1.5.6.

Results

The isolated Compound identified as Hydroxy citric acid (HCA) showed significant inhibition in obesity-related parameters like 3T3 L1 cell line study for with Insulin increases glucose uptake, metabolism and storage in adipose tissue and skeletal muscle. Glucose uptake assay study of HCA showed that an increase in glucose uptake. Molecular Docking studies results show that the hydroxy citric acid bind to the active site region of human aldose reductase (PDB 3RX2), Human

Mineralocorticoid receptor (PDB 3VHU) enzymes with good binding energy compared to standard compound of metformin.

Conclusion

The isolated compounds from *Zanthoxylum rhetsa* Linn based on results analysis of molecular docking and cell line studies compound so it can be effectively used for the treatment of Diabeties related obesity which is predicted based on results scores.

Keywords: Autodock, Hydroxy citric acid, 3T3-L1 cell lines, *Zanthoxylum rhetsa* Linn.

INTRODUCTION

Natural products such as vegetables and cooking items have a long history of being used to cure and prevent diseases. Currently, about 25% of the drugs available for treating diseases are derived from these products **[1]**.

Type-2 diabetes is a condition that can affect a person's quality of life and increase their risk factors for various health conditions **[2]**. Currently, there are various drugs available to treat this condition. Oral antidiabetic drug-like sulfonylureas, biguanides, α-glucosidase inhibitors **[3-5]** are expensive and have side effects, whereas herbal medicines are inexpensive and readily available for the treatment.

Zanthoxylum rhetsa Linn seeds belonging to the Rutaceae family have been used as food substances for thousands of years. Its high antioxidant properties **[6-9]** have made it more valuable in the research field. Recently it is used for treating various conditions like dental caries, dizziness and bloating, malaria, urinary infections, rheumatism, diuretics, stomach discomfort, and diarrhoea **[10]**.

This study aims to isolate new biomarkers from the *Zanthoxylum rhetsa* Linn seeds and to study In-vitro antidiabetic property using 3T3-L1 preadipocyte cell lines. Antidiabetic parameters like alpha-glucosidase and alpha-amylase enzymes inhibition, and In-silico molecular docking were studied.

MATERIALS AND METHODS

A. Chemicals

Analytical grade chemicals were used in this work. 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT), FBS (Fetal Bovine Serum Phosphate Buffered Saline), DMEM/F12 (Dulbecco's Modified Essential Medium (DMEM) and Ham's F-12 Medium), trypsin, EDTA, glucose, and antibiotics were procured from Fisher Scientific India and Merck Ltd Laboratories. All the other chemicals obtained from Hi media laborites India.

B. Collection of Medicinal plant

The plant *Zanthoxylum rhetsa* Linn was gathered in Ponda, Goa. The Plant Anatomy Research Center in West Tambaram validated and certified the plant.

The authentication number is PARC/2018/3834.

C. Isolation of Hydroxy Citric Acid

70 g of dry *Zanthoxylum rhetsa* Linn and 200 ml of distilled water was agitated for 30 minutes with a mechanical stirrer. Vacuum filtration was used to filter the solution. The filtrate was taken out and combined with a 1N calcium hydroxide solution and agitated with a magnetic stirrer until it achieved a pH of 7.0. The calcium salt of hydroxy citric acid precipitated were collected, filtered. Upon acid hydrolysis hydroxy citric acid salt was precipitated and then dried in a hot air oven **[11-15]**.

D. Adipocyte differentiation in 3T3-L1 cells

3T3-L1 cells were obtained from Swiss mice. 3T3-L1 cells are embryonic stem cells that can be separated into pre-adipocytes. 3T3-L1 cells were plated in 96-well plates (5 × 103 cells/well) for glucose estimation using DMEM/F12 medium with 10% FBS. The media was changed to division medium (DMEM/F12 + 2% FBS) with 10 g/mL insulin, 0.5 mM 3-isobutyl-1-methylxanthine, and 1.0 mM dexamethasone after two days of effective transformation. Dexamethasone, isobutyl methylxanthine, and insulin were administered to 3T3-L1 cells in an adjusted mixture **[16-17]**. The cells were kept in separate medium for four days, with the media being replaced every 48 hours. Following a 48-hour separation, the medium was replaced with DMEM/F12 + 2% FBS, a substance that aids in the evaluation of glucose uptake test.

i. Glucose uptake assay

The test chemicals were given to 3T3-L1 adipocyte cells at varied concentrations (10g/ml, 20g/ml, 40g/ml, and 80g/ml). Cells were introduced in triplicate to individual wells and cultured for 5 hours with and without insulin. The supernatant was discarded, and the cell lysate was utilised to calculate glucose concentration using the DNA technique. At 570 nm, the plate was read. The test samples' readings were graded by comparing them to the zero control. Using the MTT assay, the percent viability of the test compounds was determined in comparison to the control **[18-20]**.

$$\textbf{Percent Increase} = \frac{t}{c} \times 100$$

were, t - absorbance of test substance c - absorbance of the control.

E. Molecular Docking Studies

The following protein receptors were used in docking studies: Protein Data Bank was used to collect Human Aldol Reductase (PDB 3RX2) and Human

Mineralocorticoid Receptor (PDB 3VHU) receptors. The water molecules were removed. Hydroxy citric acid, which was retrieved from PubChem, was the ligand of interest. Their structures were analysed in CHEM DRAW 16.0, and the ligand files were translated to the PDB file format using CHIMERA software **[21]**.

i. Preparation of docking structures

ACD/ChemSketch 10.0 was used to create and optimise ligands, while MGLTools 1.5.6 was utilised to prepare ligand and receptor for docking. The ligands' permitted torsions were all set to flexible. Human Aldol Reductase (PDB 3RX2) and Human Mineralocorticoid Receptor (PDB 3VHU) enzyme structures in PDB. In PDB crystal structures, all heteroatoms, water molecules, and associated ligands were removed from the receptors. The receptor was configured as stiff with no flexible bonds after adding polar hydrogen and charges **[22-26]**.

ii. Docking studies

An automated docking programme, AutoDock 1.5.6, was used to perform molecular docking experiments for the chosen compounds with hydroxy citric acid. This algorithm is based on the Lamarckian Genetic Algorithm. In the drug development process, the precise interaction of bioactive substances or candidate compounds with their targets is important. AutoDock achieves this goal by combining two methods: quick grid-based energy evaluation and efficient torsional freedom search **[27-30]**.

iii. Discovery studio visualizer

Accelrys' Discovery studio visualizer generates 2D receptor-ligand interaction plots and analyses ligand-binding patterns between a protein and its bound ligands.

RESULTS

A. Isolation of hydroxy citric acid

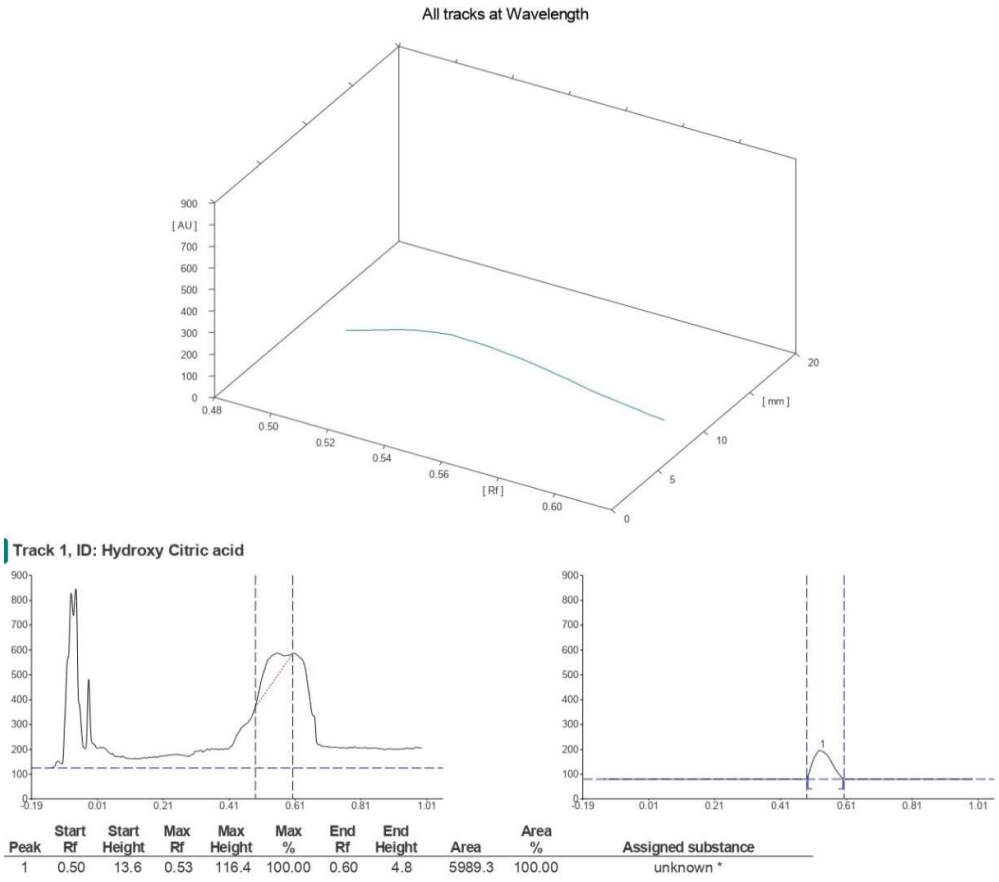
The isolated active compound confirmed by using HPTLC data reveals that positive chemical test with hydroxy citric acid.

A. Glucose uptake assay

In vitro study reveals the isolated hydroxy citric acid has antidiabetic action, which enhances glucose absorption as assessed by insulin. Anti-diabetic action was reduced glucose absorption in the absence of insulin **(Table 2)**. When compared to the low dosage (20 g/ml), the high concentration (80 g/ml) has reduced glucose absorption.

A. Docking analysis

In silico study was used to determine the inhibitory capability of a plant-derived chemical against the Human Mineralocorticoid Receptor (PDB 3VHU). Using AutoDock 4.2, the predicted binding energy of each molecule with the Human Aldol Reductase (PDB 3RX2) and Human Mineralocorticoid Receptor (PDB 3VHU) enzymes **(Table 2, Figure 2-5)**.



Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	0.50	13.6	0.53	116.4	100.00	0.60	4.8	5989.3	100.00	unknown *

Figure 1: HPTLC Spectrum of isolated compound of Hydroxy citric acid

Table 1: Antidiabetic Potential of Isolated Compound from *Zanthoxylum rhetsa* Linn on 3T3- L1 Cells by Glucose Uptake Assay

Sl.NO	Concentration (µg/ml)	Glucose Uptake percentage	
		Insulin Absent	Insulin Present
1	10	80.71429	135.2022
2	20	87.83673	162.427
3	40	73.55102	1321.6404
4	80	61.22449	117.2921

Values are represented as mean ± SEM of % of inhibition (n = 5)

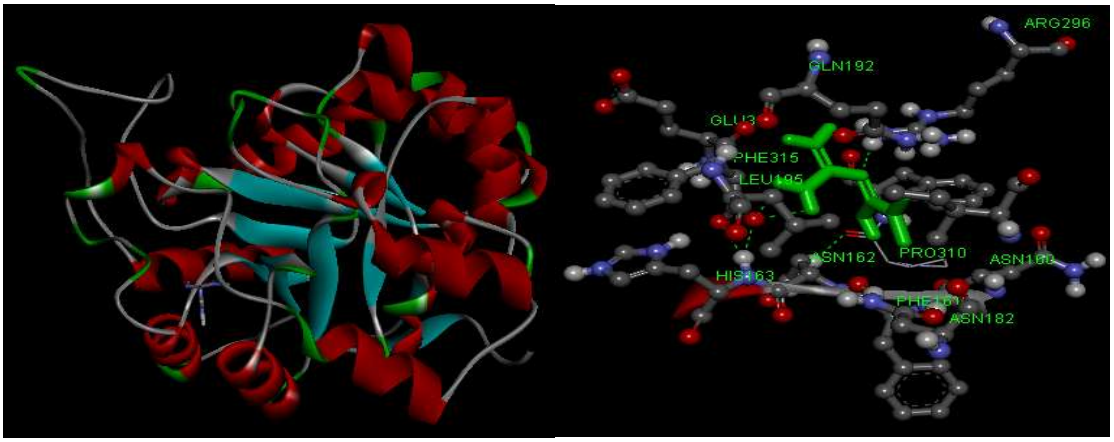


Figure 2: Metformin docked with 3RX2 and its interactions

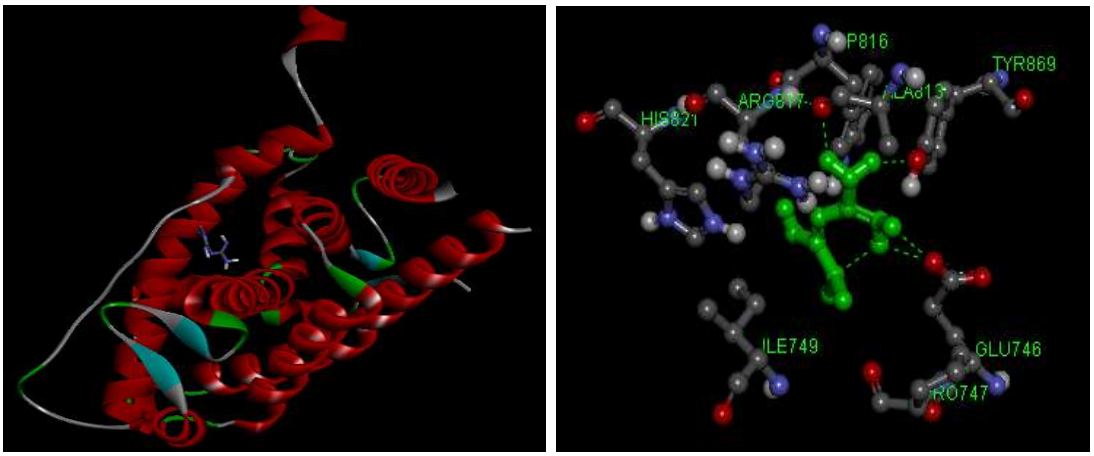


Figure 3: Metformin docked with 3VHU and its interactions

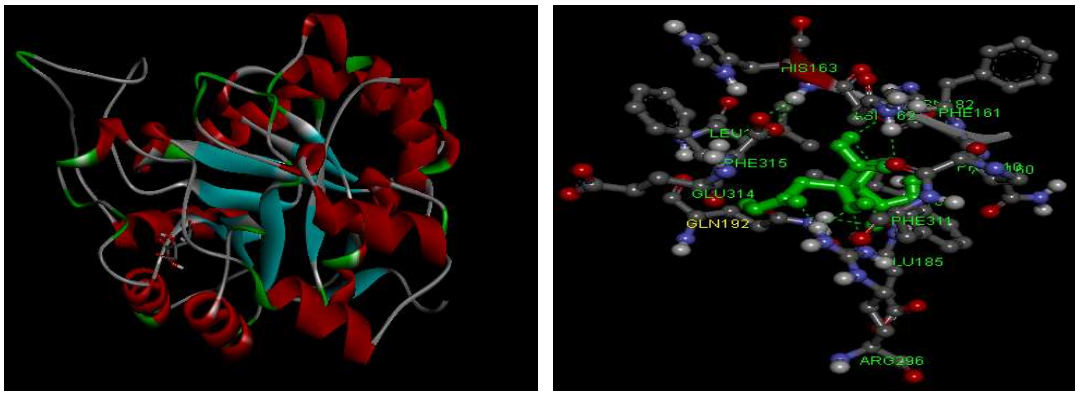


Figure 4: Hydroxycitric acid docked with 3RX2 and its interactions

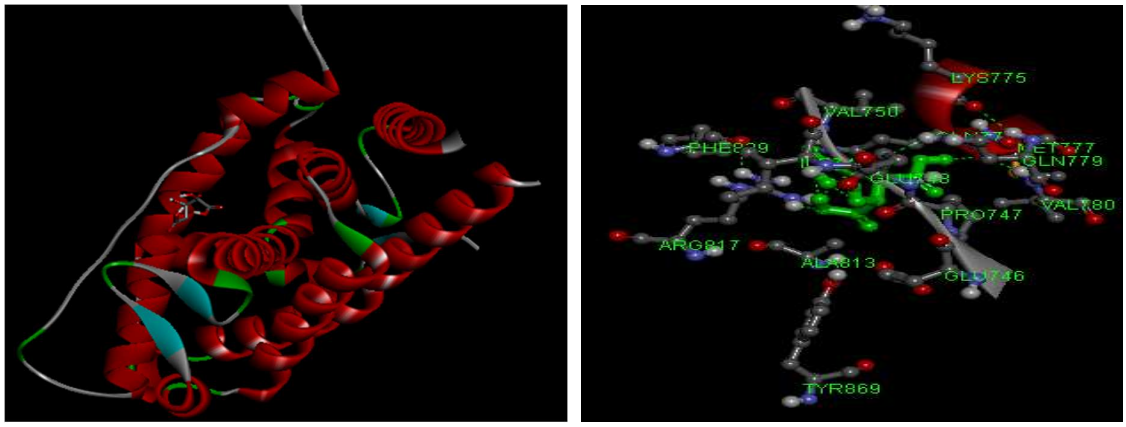


Figure 5: Hydroxycitric acid docked with 3VHU and its interactions

Table 2: Molecular docking studies of the isolated compound from *Zanthoxylum rhetsa* Linn Energy parameters obtained in Docking

S. No.	Compound	PDB ID	Binding energy	Vdw+Hb+de solv energy	Electorstatic energy	Inter molecular energy	Ligand efficiency	Number of Hydrogen bonds
1.	Metformin	3RX2	-2.46	-1.86	-0.6	-2.46	-0.27	Nil
2.	Metformin	3VHU	-4.61	-4.43	-0.18	-4.61	-0.51	Nil
3.	Hydroxy citric acid	3RX2	-1.97	-1.24	-0.23	-1.01	0.14	GLN192,ARG296
4.	Hydroxy citric acid	3VHU	-2.88	-4.34	-1.53	-5.86	-0.21	VAL750,ARG17

DISCUSSION

In this study, the effect of glucose uptake of HCA from *Zanthoxylum rhetsa* Linn seed extract was investigated on 3T3-L1 adipocytes. The results showed that HCA could significantly decrease the glucose content in the culture medium and thus elicited a hypoglycemic effect. Studies indicate that HCA elicits glycaemic control mediated through an increase in insulin-dependent receptor kinase activity, thus enhancing the insulin signaling pathway, and leads to increased glucose transporter 4 translocation and glucose uptake.

Docking studies showed that the ligands bind to human aldose reductase (PDB 3RX2), Human Mineralocorticoid receptor (PDB 3VHU) enzymes with good binding energy compared to standard. The results showed that the isolated compound from *Zanthoxylum rhetsa* Linn increases glucose uptake under in-vitro conditions. It may occur due to the presence isolated compound of hydroxy citric acid from *Zanthoxylum rhetsa* Linn or due to its effect on the receptors present in the cell membrane.

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