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Docking Studies on Ligand molecules of Coccinia indica against Human 5'-AMP-Activated Protein Kinase Catalytic Subunit α2 –A Diabetic Mellitus Target

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ABSTRACT

Docking of a ligand with the desired protein is a method of approaches for tackling the needs for drug discovery through CADD (Computer-aided drug discovery). Molecular docking is a process by which two molecules bind to each other in a 3D space where the target site, receptor are responsible for the pharmaceutical effects [20]. Aim of this work is by using Hex 8.0 dock the ligand molecules derived from the Coccinia indica such as Pectin, β-carotene, Ellagic acid, Cucurbitacin, triterpenoids with the protein kinase domain of human 5'-AMP-activated protein kinase catalytic subunit 2, which is one of the diabetic targets. Studies shows Alcohol extract and ethanol extracts of Coccinia indica root, fruit, and aerial part and leaves alone or with combination show the decrease in blood glucose level [**25**]. Diabetic Mellitus is a group of metabolic disorder represents hyperglycemia due to defects in insulin secretion, insulin action, or both [08]. Hex software reveals the docking energy value of each molecule after the docking with the target. Pectin, Ellagic acid, and Cucurbitacin follow Lipinski rule with an energy value of -172.28, -240, -321.66, and the ligands β-carotene and triterpenoids have higher molecular weight they can't be taken orally which has an energy value of -338.45 and -303.01. The present study shows the phytoconstituents derived from Coccinia indica docked with diabetic drug target for an approach to decrease the insulin secretion and decrease the development of secondary diseases.

Key words: Docking, Coccinia indica , Diabetic Mellitus , protein kinase ,Hex 8.0

I. INTRODUCTION

Diabetes mellitus is known as "Madhumeha" [24] is a common and prevalent metabolic disorder which is one among the disease leading to the death [05,06]. According to WHO 422 million people worldwide have diabetes [WHO].DM is chronic disorder characterized by hyperglycemia or high blood sugar [06], due to defects in insulin action[13].Insulin receptor (IR) present in body cells is belongs to the class of tyrosine kinase receptor, where insulin binds during blood circulation. Insulin attached to the IR mediates the absorption of glucose from blood to cells. Insulin is secreted by of pancreatic islets in response to increase in blood glucose levels [09]. DM is characterized into Type I Juvenile-onset diabetes due to lack of insulin secretion by the pancreas and Type II Adult onset diabetes due to ineffective utilization of insulin [24]. This leads to the complications of diabetes leads to development of secondary diseases affect organs such as liver, heart, kidney and lungs [19].In this study we are going to aim AMPK as target for anti diabetic treatment approaches. AMPK is AMP-activated protein kinase is a biomarker for insulin sensitizing activities used in anti diabetic drugs [11]. AMPK is an important regulatory protein that stimulates glucose transport and fatty acid oxidation, AMPK are associated with lowering blood glucose levels. AMPK is a phylogenetically conserved serine/threonine kinase exist in heterotrimer consist of catalytic α and regulatory β and γ subunits [07].

Fig 1 depicts the Two or three genes that are encoding each subunit which will make 12 combinations of Heterotrimeric combinations.α_1 and α_2 are isoforms of α subunit, contain kinase domain in N-terminal half and C-terminal region will form complex with β and γ subunits. α_1 is predominantly cytoplasmic and α_2 consist of nucleus of all type of cells including pancreatic β cells, neurons and skeletal muscles. β Subunit contains two conserved regions, located at central and C-terminal region, required to form αβγ complexes regulated by AMP recognized to be glycogen binding domain.δ (δ_1, δ_2, δ_3) subunits contain variable N-terminal regions followed by tandem repeats termed as Bateman as CBS motif, each of which binds to one molecule of AMP. Mutation in this binding will affect binding and activation of AMP.ATP can also binds to this region, higher concentration of ATP will decrease the activation of AMPK complex [28].

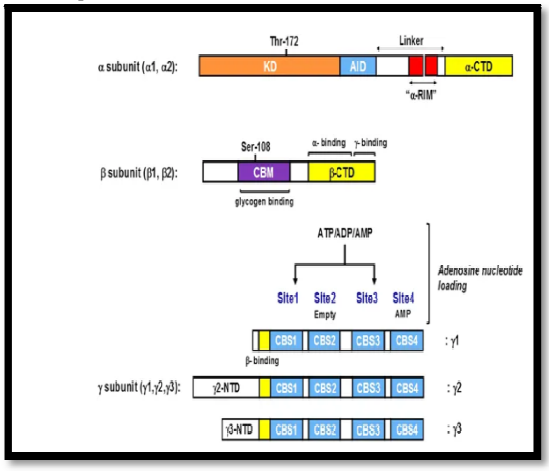

Fig. 1 Gene encoding the structure of AMPK

A. *Regulation of Ampk:*

Metabolic stress will inhibit ATP production or ATP consumption, which will increase cellular ADP: ATP ratio, Adenylate kinase will amplifies much increase in the AMP: ATP ratio with consequent activation of AMPK activation .During Exercise or contraction, Physiological stress that activates AMPK in skeletal muscle.AMP concentration is lower than ADP or ATP in unstressed cells, ADP: ATP ratio rises during stress conditions, due to displacement of Adenylate kinase reaction towards AMP, AMP should therefore be able to compete ATP and ADP at AMPK-γ subunit. Binding of AMP or ADP to AMPK-γ subunit leads to the conformational change and Phosphorylation of Thr-172 by upstream kinases, inhibit dephosphorylation by phosphotases. Phosphorylation of Thr-172 in α subunit causes greater than 100 fold activation of AMPK, Phosphorylation of Thr 172 in the α subunit is the principal event required for full activation of AMPK. Binding of AMP causes allosteric activation. Small increase in ADP: ATP or AMP: ATP will increase AMPK activity[28, 07].Serine/ Threonine kinase LKB1 (Liver kinase-B1) is responsible for the Phosphorylation of upstream kinase Phosphorylation. Intracellular increase in the Ca^{2+} also activates AMPK by Ca^{2+}/calmodulin dependent kinase 2, Camkkβ.ATP binding will inhibit all the three mechanisms [02, 28].

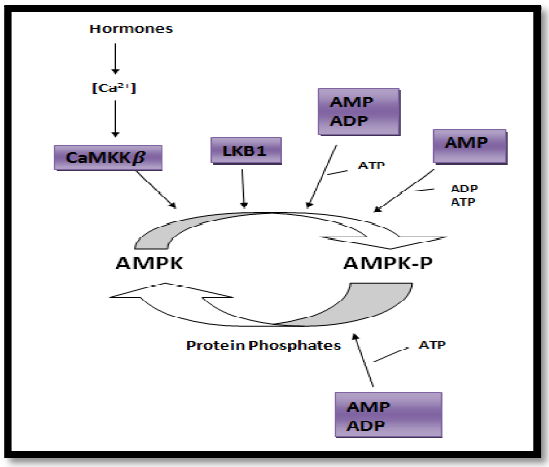

Fig. 2 Ternate mechanism of AMPK activation by changes in cellular energy status

B. *Diet and Diabetes:*

India have listed around 45000 plant species out of 7500 species of plants have medicinal importance. History showed that medicinal plants have been used in traditional healing and treatment of diabetes. This is because such herbal plants have hypoglycemic properties and other beneficial properties [04].Plant constituents play an important role as alternative with low cost and less side effects. The beneficial part of plant constituents in diabetes is Hypoglycemic herbs increase insulin secretion, enhance glucose uptake by adipose or muscle tissues and inhibit glucose absorption from intestine and glucose production from liver[10].Coccinia indica (Cucurbitaceae) is commonly known as Ivy gourd which origin lies at Africa, India and Asia [23].Different part of this plant (roots, leaves, and friuits) used as folk medicines for several diseases such as Jaundice, diabetes, wound healing, ulcers, stomach ache, skin disease, fever, asthma, cough. Leaf constituents possess anthelmintic activity, antimicrobial activity, anti- hyperglycemic activity, anti-inflammatory, and analgesic and hepatoprotective activity. Chemical constituents in plants are Resins, alkaloids, fatty acids, flavonoids, and proteins , methanol extracts form fruits contains Alkaloids, steroids, tannins, saponins, β-carotene ,ellagic acid, phenols, glycosides, lignans and triterpenoids. Roots contains triterpenoid, saponincoccinioside, flavonoid glycoside ombuin 3-o-arabino furanoside, Lupeol , β-amyrin and β-sitosterol and Stigmast-7-en-3-one [12].

In this paper we are trying to dock above plant constituents present in coccinia indica with one of the Anti-diabetic target of protein kinase domain of human 5'- AMP-activated protein kinase catalytic subunit α_2 with Hex .8.0 Software.

Table 1 Selective phytochemicals derived from coccinia indica selected for docking with protein kinase domain of human 5'-AMP-activated protein kinase catalytic subunit α_2

PECTIN		ELLAGIC ACID
• Pectins are biopolymers, because of their structural diversity it is applied in various fields. • Bioactive pectin polysaacharide have pharmacological applications include anti-inflammatory, Hypoglycemic, antibacterial, antitumor and immuno regulatory. • Orally administered pectin extract leads to hyperglycemia by reduction in blood glucose and an increase in the liver glycogen as a result of increase in hepatic glycogen synthetase activity and corresponding reduction in phosphorylase activity.[29,01]	• **β-CAROTENE** is a member of carotenoids that are under a group of phytochemicals. • Because of its extended structural system with double bonds they are strong antioxidants so that decrease the risk of development of type 2 diabetes. • Increase intake of orange fruits such as pumpkin, papayas, cantaloupe, and orange root, vegetables like carrots and sweet potatoes, and in leafy vegetables like spinach, broccoli beta carotene is masked by chlorophyll reduce the risk associated with secondary diseases.[21,14]	• It is discovered in 1831 and has a molecular weight of 302.197g/mol. these compounds are usually found in fruits, vegetables, nuts, and wine. • It is a bioactive polyphenol having antioxidant properties. it plays an antidiabetic activity on cells of the pancreas, stimulating insulin secretion and decreasing glucose tolerance. ellagic acid activity in cells, increasing in cell number, decreasing blood glucose, cell morphology, and increasing antioxidant status.[03]

CUCURBITACIN

- Cucurbitacins are a group of bitter-tasting, triterpene plant which is oxygenated containing plants were early recognized to have biological values.
- Hypoglycemic activity of Cucurbitacin-B is due to the potential induction of novel protein CYP450.
- Cucurbitacin-B can be used as an effective therapy for the postprandial hyperglycemia with minimal side effects.[1,17]

TRITERPENOIDS

- Triterpenoids constitute a large structurally group of natural compounds derived from active isoprenes. they can inhibit enzymes involved in glucose metabolism, prevent the development of insulin resistance and normalize plasma glucose and insulin levels[16]
- Anti-diabetic property and hypoglycemic property of alloxan-induced rats is due to enhance glucose utilization by peripheral tissues and enhancement of insulin effect in the plasma due to the secretion of insulin by β-cells of islets of Langerhans or its release from bound insulin.[27].

II. MATERIALS AND METHODS:

A. Protein Preparation (Target preparation):

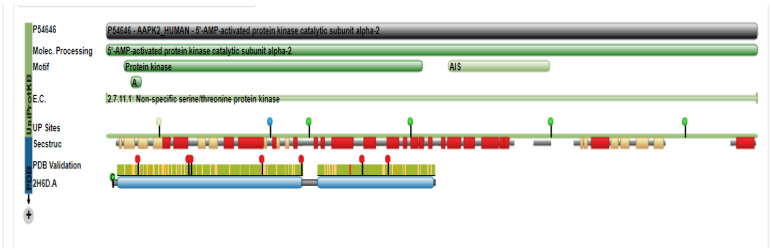

Fig. 3 Protein domain structure of 5'-AMP-activated protein kinase

The PDB is the major repository of protein structures. This databank stores three dimensional atomic coordinates of proteins and nucleic acids obtained through X-Ray crystallography and NMR experiments (RCSB PDB).The structure of protein kinase domain of human 5'-AMP-activated protein kinase catalytic subunit α_2 (2H6D) obtained from RCSB PDB. Complexes such as Non essential water molecules and hetero atoms are removed and Energy minimization is carried out using SWISS PDB VIEWER [26]. Figure 3 depicts the domain structure of 5'-AMP-Activated protein kinase.

B. Ligand preparation:

Ligands such as Pectin, Ellagic acid, β-carotene, Triterpenoids, Cucurbitacin retrieved from pubchem.ncbi.nlm.nih.gov in SDF format and converted them into PDB format using Corinar 3D converter. Lead validation is done by using SwissADME website used to predict ADME parameters, pharmacokinetic properties, and drug like nature of selected ligands. These ligands are opened in Hex 8.0.0 for docking studies.

C. Ligand- Receptor Docking:

Hex 8.0 software calculates protein ligand docking by assuming ligand is rigid, it uses spherical polar fourier (SPF) correlations to accelerate the calculations and one of the few program uses graphic interface. It can superpose pairs of molecules using only their 3D shapes [30].

Phytochemicals such as triterpenoids and cucurbitacin (triterpenes), pectin (heteropolysaccahride), ellagic acid (polyphenol), β-carotene (carotenes) derived from Coccinia indica are docked with protein kinase domain of human 5'-AMP-activated protein kinase catalytic subunit as target for antidiabetic activity of above plant constituents by following above parameters.

Table 2 parameters followed for docking in Hex 8.0 software

Correlation Type	Shape only
FTT mode	3D Fast
Grid dimension	0.6
Receptor range	180
Ligand Range	180
Twist Range	360
Distance Range	40
Ligand Molecule	Virchole
Drug molecule	Ball and stick

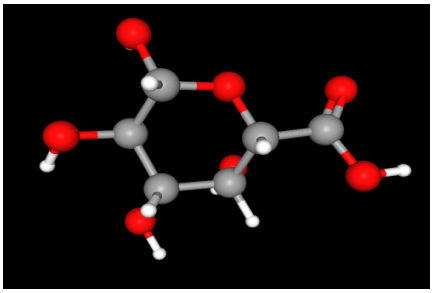

Fig. 4 Structure of pectin retrieved from pubchem

Fig. 5 Structure of Ellagic acid retrieved from pubchem

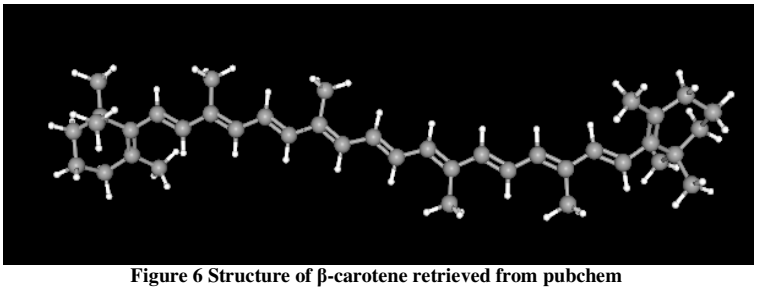

Figure 6 Structure of β-carotene retrieved from pubchem

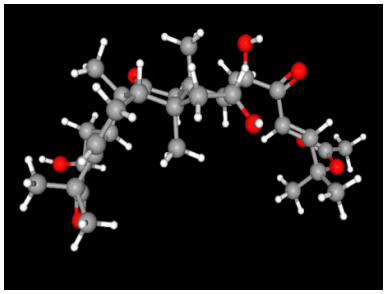

Fig. 7 Structure of Cucurbitacin B retrieved from pubchem

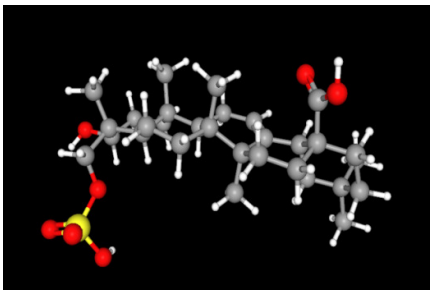

Fig. 8 Structure of triterpenoids retrieved from pubchem

III. RESULT AND DISCUSSIONS

Docking is used to predict the binding of small candidate drugs to their protein targets in order to predict the affinity and activity of the small molecule. Docking plays an crucial role in drug discovery. Candidate ligands used in our paper are plant constituents present in Coccinia indica such as carotenoids (β-carotene), Polyphenols (Ellagic acid), Terpenoids (Triterpenoids, cucurbitacin), polysaccharide (Pectin) is docked with Target protein kinase domain of human 5'-AMP-activated protein kinase catalytic subunit.

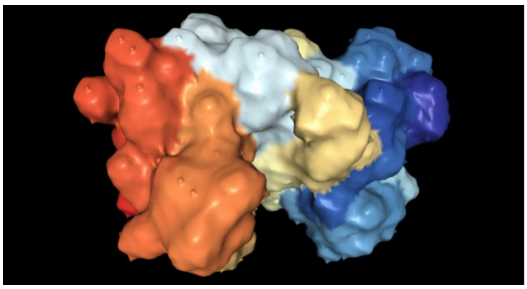

Fig. 9 Structure of protein kinase domain of human 5'-AMP-activated protein kinase catalytic subunit

Protein kinase domain of Human 5'-AMP-Activated protein kinase catalytic subunit activated by phosphorylation of Thr-172.It is about 276aa of sequence length. The energy minimization is done by removing water molecules and adding hydrogen using SWISS PDB VIEWER. The final energy value

E= 12041.845KJ/mol. Computations were done with GROMOS 43B1 parameters set without reaction field in SWISS PDB VIEWER.

LEU	A 276	0.421	3.595	4.296	1.729	-46.57	1.44	0.0000 // E= -35.090
PHE	A 277	1.488	1.656	1.065	1.562	-53.55	34.83	0.0000 // E= -12.961
PRO	A 278	0.563	14.881	20.661	1.578	-13.13	-10.86	0.0000 // E= 13.694
OXT	A 278	0.000	0.000	0.000	0.000	-0.71	11.70	0.0000 // E= 10.988
KJ/mol		265.902	926.419	1376.332	297.777	-8317.60	-6590.68	0.0000 // E= -12041.845

Fig. 10 Energy minimization of selcted target in Swiss PDB Viewer

Ligands are phytochemicals under the categorisation such as Polyphenols, Carotenoids, polysaccharides, Terpenes that binds with target for diabetes and decrease the consequences of further secondary diseases.

Table 3 Energy scored for docking of each ligands with target protein kinase domain of human 5'-AMP-activated protein kinase catalytic subunit

MOLECULE	MOLECULAR WEIGHT	HYDROGEN BOND ACCEPTOR	HYDROGEN BOND DONOR	$LogP_3$	GI absorption	Lipnski
Pectin($C_6H_{10}O_7$)	194.14 g/mol	7	5	-2.34	low	yes
Ellagic acid ($C_{14}H_6O_8$)	302.19 g/mol	8	4	1.10	high	yes
Cucurbitacin B ($C_{32}H_{46}O_8$)	558.70 g/mol	8	3	2.64	low	yes
β Carotene ($C_{40}H_{56}$)	536.87 g/mol	0	0	13.54	low	no
Triterpenoids	552.76 g/mol	7	3	5.88	low	no

Docking is an essential tool for calculation of binding interactrions of ligands to the protein target. Docking studies predicts the molecules fits into the active site and also minimum energy for interaction. (15) Docking determines the drug-ligand complex with lowest energy and also finds the ligands that fits the target with best scores.(22).

C. Interaction studies of docking:

Phytoconstituents like polyphenols, polysaccharide, carotenoids and terpenes binds with protein kinase domain of human 5'-AMP-activated protein kinase catalytic subunit α_2.All the ligand bindings values are negative, i.e, all the compounds shows greater affinity towards the target. So these constituents can be effectively used in anti diabetic studies and decrease in the secondary disease emergence. Energy values are as follow as in Table 4. The larger negative value is obtained for the ligands as follows: β-carotene with target (-**338.45**), Cucurbitacin with target (-**321.66**), Triterpenoids with target (**-303.01**),Ellagic Acid with target (**-240**), Pectin with target **(-172.28).** β-carotene have the largest interaction with protein kinase domain of human 5'-AMP-activated protein kinase catalytic subunit α_2.The β-carotene and Triterpenoids due to higher molecular weight they violates lipnski rule, which cannot take orally.

TARGET	LIGAND	RMS	E-Total	E-shape
protein kinase domain of human 5'-AMP-activated protein kinase catalytic subunit	Pectin	-1	-172.28	172.28
	Ellagic Acid	-1	-240	240
	β-Carotene	-1	-338.45	338.45
	Cucurbitacin	-1	-321.66	321.66
	Triterpenoids	-1	-303.01	303.01

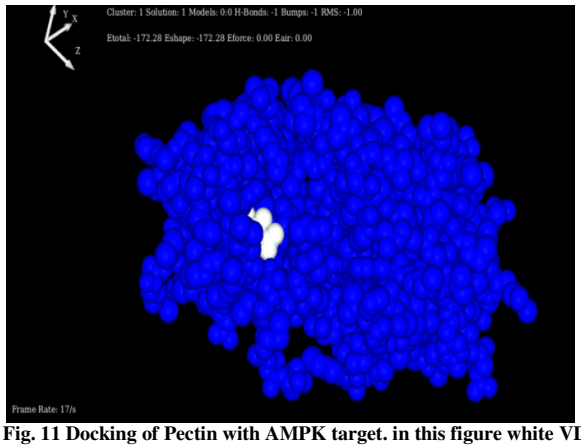

Fig. 11 Docking of Pectin with AMPK target. in this figure white VDW sphere model type is pectin and blue is Target with peptide thickness of 0.10

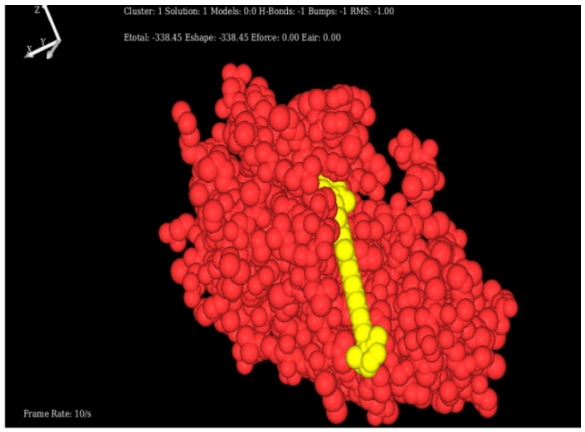

Fig. 12 Structure of docking of Beta carotene with Ampk with beta carotene on VDW sphere model type with peptide thickness of 0.10

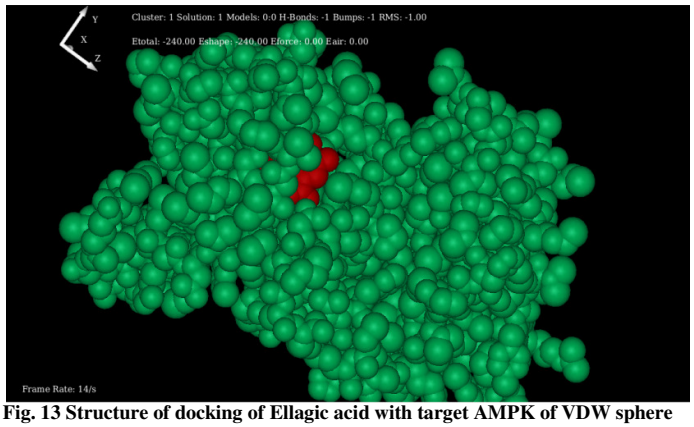

Fig. 13 Structure of docking of Ellagic acid with target AMPK of VDW sphere model type in Hex 8.0

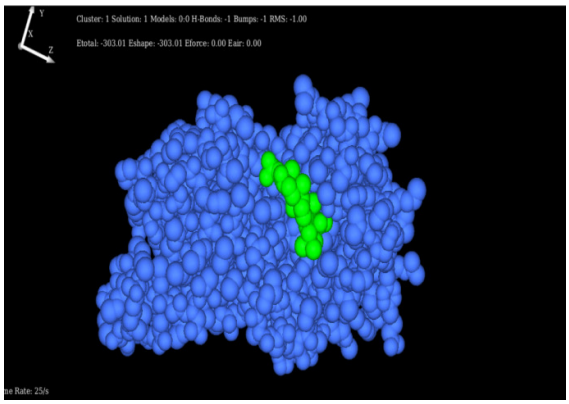

Fig. 14 Structure of docking of tritrpenoids with target AMPK of VDW sphere model type in Hex 8.0

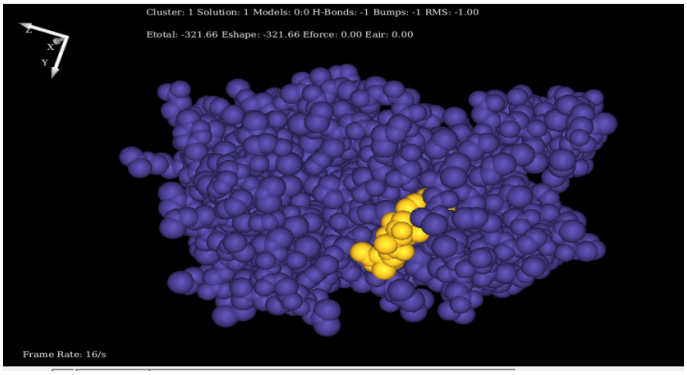

Fig. 15 Structure of docking of Cucurbitacin B with target AMPK of VDW sphere model type in Hex 8.0

IV. CONCLUSION

The results of the current study have revealed that the compounds of the selected plant Coccinia indica contains a potential inhibitor for human 5'-AMP-activated protein kinase catalytic subunit α2 a Diabetic target. Thus validated the possibility of coccinia indica plant extract as a new alternative to existing diabetic approaches.

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