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In Silico Docking of Selected Compound from *Cardiospermum halicacabum* Linn. Leaf against Human Hepatocellular Carcinoma (HepG2) Cell Line

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ABSTRACT

The present molecular docking study stands useful for the design and development of novel compound having better inhibitory activity against the selective proteins of human hepatocellular carcinoma cell line. The docking scores were highest for Transferrin with –6.13 kcal/mol with the stronger interaction followed by Plasminogen (-1.69 kcal/mol having least score, the LogP, lower hydrogen bond counts, confirming the capability of Stearic acid for binding at the active site of the receptor. This potential drug candidate can further be validated by wet lab studies for its proper function.

Keywords: Stearic acid, HepG2 cell line and Hex.

1. INTRODUCTION

Cancer continues to be one of the major causes of death worldwide and only modest process has been made in reducing the morbidity and mortality of this dreadful disease. Extensive preclinical and clinical research has led to substantial progress in understanding the multistep nature of the prolonged tumorigenesis process. This understanding has led to the realization that most human malignancies should be fought on multiple fronts. Thus, in addition to cancer therapy, cancer prevention has become an important approach to control cancer [1, 2]. Common prevention strategies include avoiding exposure to known cancer causing agents, enhancement of host defense mechanisms against cancer, lifestyle modifications and chemoprevention [3].

Cancer chemoprevention uses agents that slow the progression of, reverse or inhibit carcinogenesis in healthy subjects, thereby lowering the risk of developing invasive or clinically significant disease [4]. Consequently, an effective chemopreventive agent should intervene early in the process of carcinogenesis to eliminate premalignant cells before they become malignant or protect normal cells from undergoing transformation. The latter strategy is more difficult to implement since otherwise healthy individuals would perhaps need a lifetime of exposure to a particular chemopreventive agent to achieve efficacy. It could be reasonably argued that the same benefit could be derived from avoiding exposure to known cancer causing agents and consuming a balanced diet. The former approach is more practical in short-term interventions targeting premalignant lesions or preventing second primary tumors [2]. Thus far, the design of most human cancer chemoprevention trials would suggest that this methodology is currently favored [3].

Hepatocellular carcinoma (HCC) is the primary form of human adult liver cancer and it is the fifth most commonly occurring forms of cancer worldwide. It is known to occur widely in China, most parts of South East Asia and South Africa. The incidence of HCC is on the rise with one million new cases being diagnosed

every year and an equal number of deaths occurring [5].

Some of the options available for treatment are orthoptic liver transplantation, surgical resection and local destruction. The recurrent rate continues to be high with 50% recurrence in two years. The other option available is chemotherapy and treatment with interferons and hormones. This also remains a challenge as HCC is highly resistant to systemic treatments. The search for more active and specific treatment for liver cancer is continuing and many molecular compounds derived from plants have shown promise [6].

Stearic acid is a saturated fatty acid. The salts and esters of stearic acid are called stearates. As its ester, stearic acid is one of the most common saturated fatty acids found in nature following palmitic acid [7]. Stearic acid is mainly used in the production of detergents, soaps and cosmetics such as shampoos and shaving cream products. Stearic acids used along with caster oil for preparing softeners in textile sitting [8].

In silico modeling is the find-a-way around for the traditional drug testing compounds, synthesized in time consuming multi step process against biological screens. It is the new loom to clinical chemistry for the optimization of screening and testing by means of the observation on particular compound [9]. Computational biology and bioinformatics have the potential not only of speeding up the drug discovery process, thus reducing the costs, but also of changing the way drugs are designed. Rational Drug Design (RDD) helps to facilitate and speed up the drug designing process, which involves variety of methods to identify novel compounds [9, 10]. One such method is the docking of the drug molecule with the receptor (target). The site of drug action, which is ultimately responsible for the pharmaceutical effect, is a receptor [11].

Nowadays, the use of computers to predict the binding of libraries of small molecules to known target structures is an increasingly important component in the drug discovery process [12, 13]. There are wide ranges of software packages that are available for the conduct of molecular docking simulations like, AutoDock, GOLD, FlexX *etc* [13]. AutoDock 4.2 has been widely used for virtual screening, due to its enhanced docking speed as stated they Schames *et al.* (2004) [14]. Its default search function is based on Lamarckian Genetic Algorithm (LGA), a hybrid genetic algorithm with local optimization that uses a parameterized free-energy scoring function to estimate the binding energy. Each docking is comprised of multiple independent executions of LGA and a potential way to increase its performance is to parallelize the aspects for execution [15]. Docking of small molecules in the receptor binding site and estimation of binding affinity of the complex is a vital part of structure based drug design [16].

Cardiospermum halicacabum Linn. is a creeping, glabrous, succulent herb distributed through-out India has been used as a traditional Ayurvedha medicine for centuries for the treatments of several diseases and disorders. In view of this, the present study is designed to identify the protein present in the HepG2 cell line like plasminogen and transferrin both are the secrete the Hepatitis B, to analyze the domain and active sites, to assess the chemical and physical properties of the protein (Plasminogen and Transferrin), to analyze the potentiality of the therapeutic agents in terms of their properties, to perform Docking of the proteins with a selective compound of stearic acid present in the *C. halicacabum* and to evaluate the compound docking and active site binding.

2. MATERIALS AND METHODS

2.1 Structure Retrieval of protein

Database similarly searches are one of the most important steps in analyzing a sequence. If the query sequence has a similar copy already in the database, a search will quickly reveal this fact. If a similarly of sequence or structure is found from another species, then they may be homologous (*i.e.*, sequence that descended from common ancestral). This will pave a way for further analysis of the query sequence. The structure homologues for a given protein sequence query is searched against SwissProt and PDB. Graphical visulation of protein was done using RasMol.

2.2 Pub Chem Compound

The PubChem Substances Database contains descriptions of chemical samples, from a variety of sources, and links to PubMed citations, protein 3D structures, and biological screening results that are available in PubChem Bio Assay**.** If the contents of a chemical sample are known, the description includes links to PubChem Compound.

2.3 Docking Hex (version 5.0)

Hex is an interactive protein docking and molecular super position program, written by Dave Ritchie. Hex understands protein and DNA structures in PDB format, and it can also read small-molecule SDF files. As of October 2013, there have been about 33,000 downloads. Hex will run on most Windows-XP, Linux and Mac OS X PCs. The recent versions now include CUDA support for Nvidia GPUs.

On a modern workstation, docking times range from a few minutes or less when the search is constrained to known binding sites, to about half an hour for a blind global search (or just a few seconds with CUDA). On multi-processor Linux systems, docking calculation times can be reduced in almost direct proportion to the number of CPUs and GPUs used. The calculations can be accelerated by using an optional disc cache (strongly recommended) of about 1 GB of disc space. The molecular docking of ligand, stearic acid was done against transferrin and plasminogen proteins to find out the efficacy of the ligand against the two proteins.

2.4 Visualization of Protein using PyMol Viewer

The PyMol software interactively displays molecular models and creates publication quality images. A `ribbon drawing' is featured here. Space-filling, ball-and-stick representations, molecular surfaces, density map contours, and crystal packing diagrams, and movies are also supported.

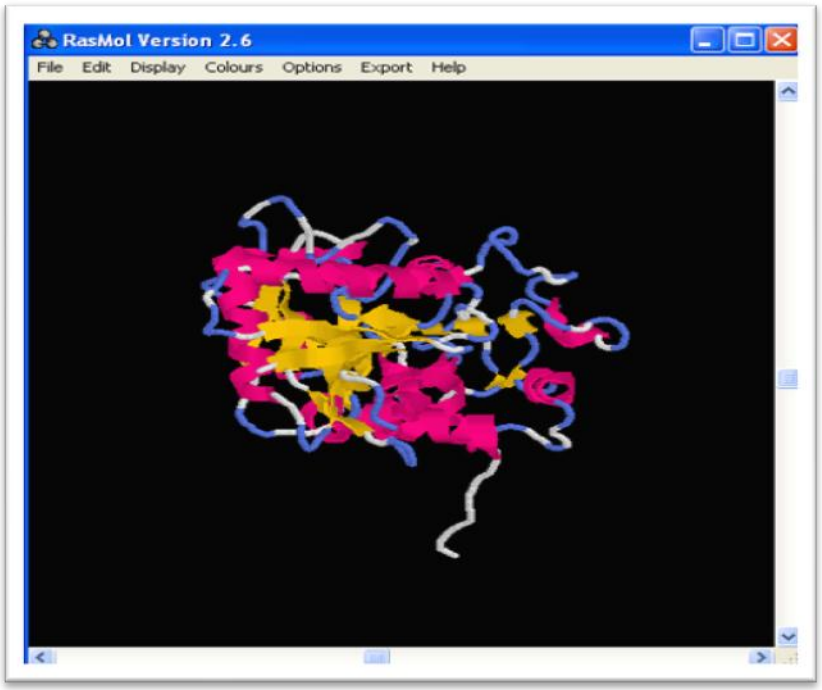

Figure 1: Visualization of Transferrin protein using Rasmol Tool

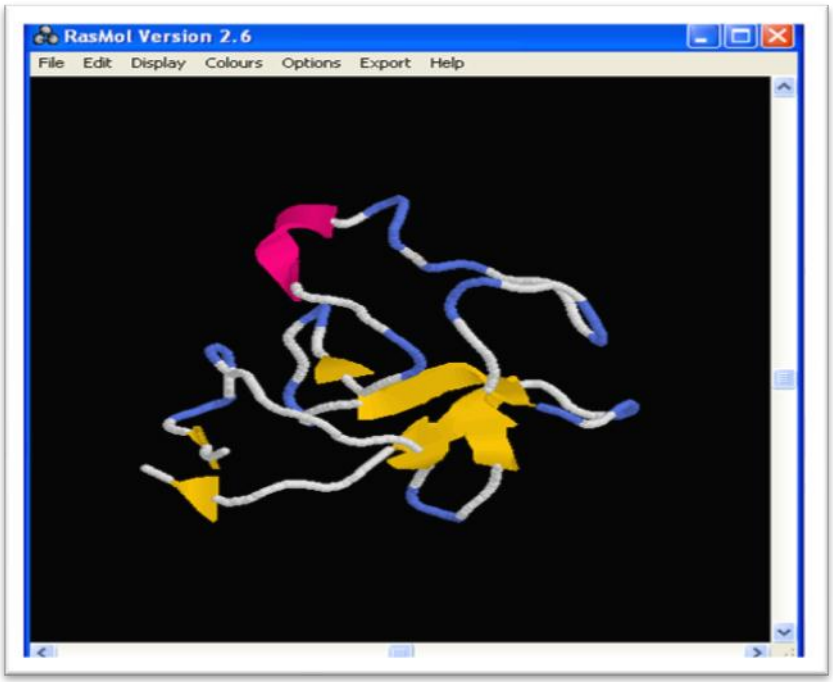

Figure 2: Visualization of Plasminogen protein using Rasmol Tool

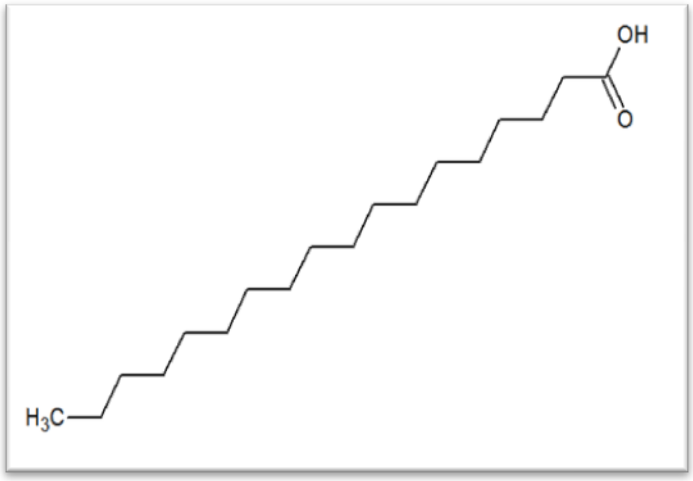

Figure 3: 2-D Structure of Stearic acid.

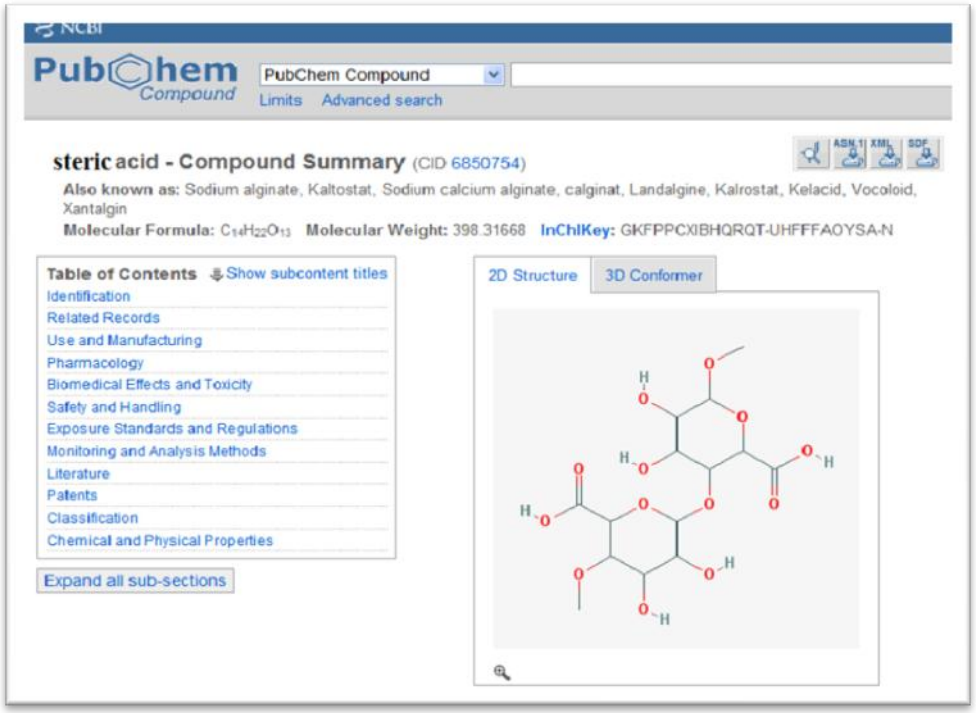

Figure 4: PubChem Compound

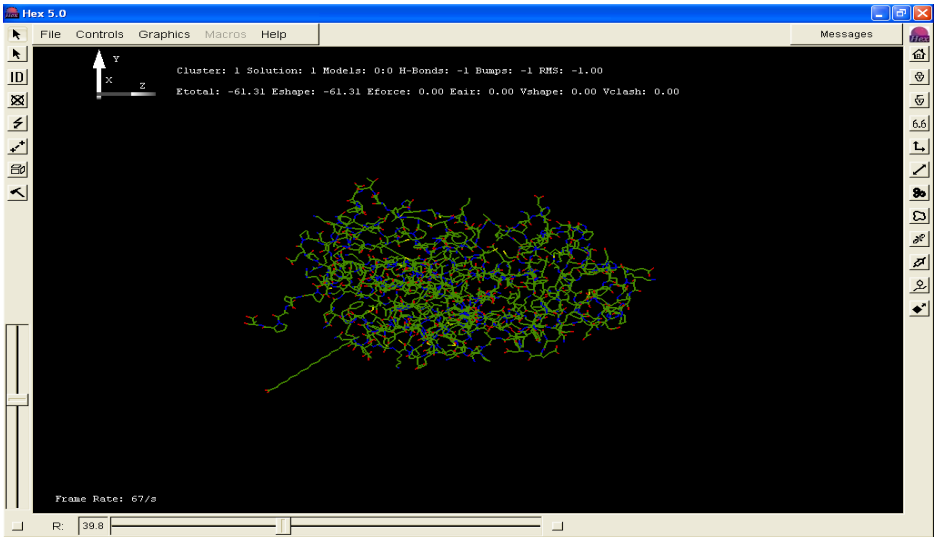

Figure 5: Hex output for Serotransferrin (Transferrin) with Steric Acid.

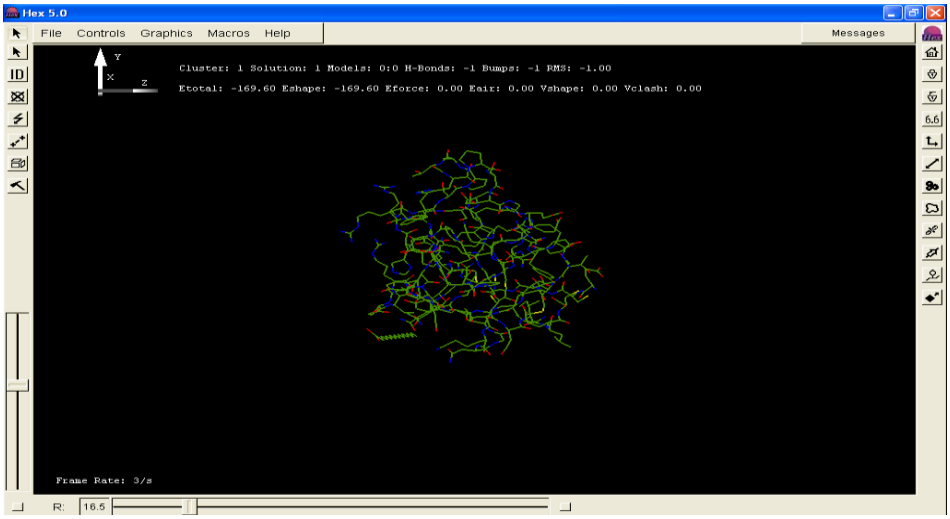

Figure 6: Hex output for Plasminogen with Steric Acid.

Table 1: Chemical Information of Steric Acid.

Name of the compound	Alternative name	Molecular weight	Molecular formula	XLogP3	H-Bond Donor	H-Bond Acceptor
Steric acid	Octadecanoic acid,Stearophanic acid, n-Octadecanoic acid, 57-11-4, Pearl stearic, Stearex Beads, Cetylacetic acid	284.47724 [g/mol]	$C_{18}H_{36}O_2$	7.4	1	2

3. RESULTS AND DISCUSSION

3.1 Retrieval of Protein Sequence from SWISS-PROT Database

For molecular docking studies of proteins viz., transferrin and plasminogen, against the ligand, stearic acid, the proteins were downloaded from SWISS-PROT database. HepG2 cell line protein structures were derived from PDB and used as a target for docking simulation are shown in the Fig. 1 and 2.

3.2 Structure Elucidation of Stearic Acid

For molecular docking studies, first the 2-D (Fig. 3) and 3-D structure of stearic acid was downloaded from ChemSketch database. Likewise the compound summary of stearic acid was obtained from PubChem database (Fig. 4). The compound details selected from the literature as shown in the Table 1.

3.3 Setting Docking Score

The docking scores were set in the Hex (Version 5.0) Tools and finally confired for docking of the Steric acid ligand against Transferrin (Fig. 5) and Plasminogen (Fig. 6). Once the scores were set and final confirmation was given the Hex (Version 5.0) software docks the Stearic acid ligand against Transferrin and Plasminogen, and the docking results are given below in Table 2 and Table 3.

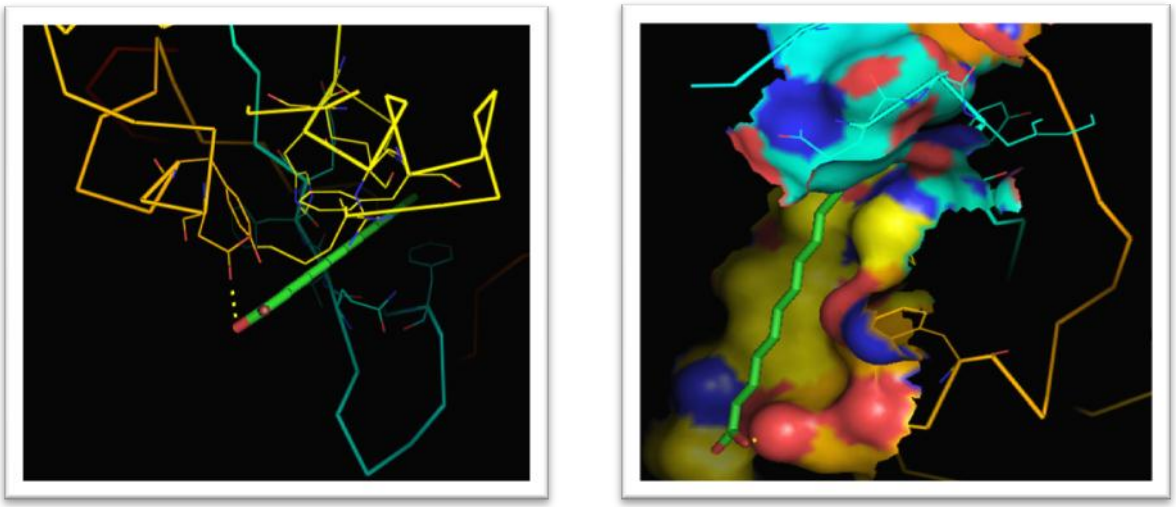

Figure 7: Visualization of docked transferrin complex using PyMol Viewer

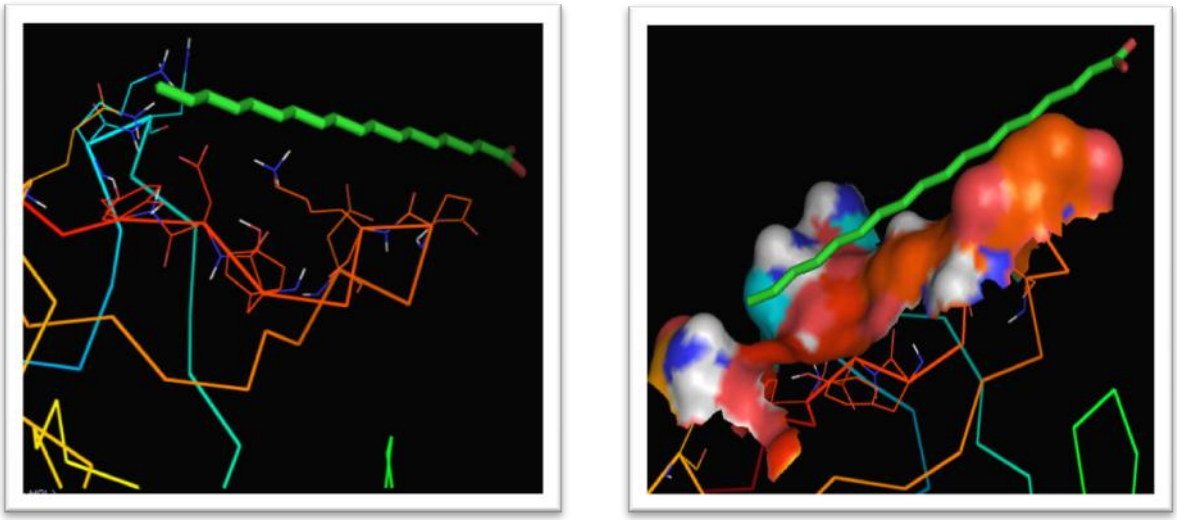

Figure 8: Visualization of docked plasminogen complex using PyMol Viewer

Table 2: Docking of Steric acid ligand against transferrin of HepG2 cells.

Transferrin		Steric Acid	RMS	Docking Score (Kcal/mol)	Distance (Å)
Residue	Atom	Atom			
ASP 236	0D2	0	-1.00	-6.13	2.17

Table 3: Docking of Steric acid ligand against plasminogen of HepG2 cells.

Plasminogen with Steric Acid		
RMS	Docking Score (Kcal/mol)	Interaction
-1.00	-1.69	Nil

3.4 Visualization of Docked Complex using PyMol Viewer

The docked complex when viewed by Pymol viewer, showed that the ligand docked only with transferrin protein (Fig. 7). On the other hand, the ligand did not have any interaction with Plasminogen protein (Fig. 8).

In silico docking study revealed the interactions between ligand and HepG2 cell line protein in order to calculate the minimum binding energy (kcal/mol) between them. In this Stearic acid showed the docking score of - 6.13 kcal/mol with Transferrin protein and the Plasminogen showed the least score of -1.69 kcal/mol with no docking interactions.

The interaction of Serotransferrin (Transferrin) with Stearic acid forms 1 hydrogen bond with energy -6.13 kcal/mol but there is no interaction between Plasminogen and Stearic acid. This result shows that there is a presence of binding site between the Serotransferrin (transferrin) and Stearic acid. The docking is also valid by the formation of hydrogen bond between them.

From the above docking results, Stearic acid docks well to the Transferrin responsible for disease and is said to be the best compound. The result of Lipinski rule suggests the analysed compound as best therapeutic drug. Docking study and *in silico* toxicity results proves the application of compounds as potential and natural therapeutic agents to treat disease.

To elucidate the medicinal properties of *C. halicacabum*, especially the active component *viz.,* steric acid, there is a need for further investigation that will pave a way for finding this herbal resource as a medicine to control hepatocellular carcinoma as well as for different types of cancers in future

4. CONCLUSION

In this study, the molecular docking was done to explore the binding mechanism and to correlate its docking score with the activity of Stearic acid compound. The results of our present study can be used for the design and development of novel compound having better inhibitory activity against several type of cancer. This potential drug candidate can further be validated in wet lab studies for its proper function.

5. Acknowledgements

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