**Journal of Food Technology Research**
*2016 Vol. 3, No. 1, pp. 12-22*
*ISSN(e): 2312-3796*
*ISSN(p): 2312-6426*
*DOI: 10.18488/journal.58/2016.3.1/58.1.12.22*


# "EVALUATION OF PHYTOCHEMICAL CONSTITUENTS OF THE FRUITS OF *CUCUMIS SATIVUS* LINN. FOR THEIR HEPATOPROTECTIVE ACTIVITY BY MOLECULAR DOCKING STUDIES"

**Subbarayan Bothi Gopalakrishnan[1†] --- Thangaraj Kalaiarasi[2] --- Shanmugam Gnanendra[3]**
[1]*Senior Professor of Chemistry & Controller of Examination, Noorul Islam University, Kumaracoil, Kanyakumari District, Tamil Nadu, India*
[2]*Assistant Professor of Chemistry, Kalasalingam Academy of Research and Education, Anand Nagar, Krishnankoil, Tamil Nadu, India*
[3]*Associate Professor of Biotechnology, Mahendra Arts and Science College, Kalippatti, Namaakkal, Tamil Nadu, India*

## ABSTRACT

*Cucumis sativus Linn.(Fam. Cucubitaceae) is commonly known as "Vellari" in Tamil, "Cucumber" in English and "Sakusa" in Sanskrit. Cucumis sativus fruit is shown to possess various activities such as ameliorative, hypoglycemic, hypolipidemic, carminative and ameliorative and antacid properties. It is used in Cosmetics as treatment for skin-inflammation and skin protectant. Pulp of the fruit is useful in dysentery, diarrhea, dropsy, piles and leprosy. It is also used as a liver tonic. Hepatocellular carcinoma is a metabolic disorder which is emerging as a severe problem and is a disease involving liver disorder. In the present study, six phytochemical constituents isolated and identified from the ethanol extract of the fruits of Cucumis sativus Linn. by GC-MS analysis have been screened for inhibitory activity against Hepatitis B X and Heme oxygenase I using molecular docking studies. The binding affinities of the Phytochemical constituents were compared with that of the known hepato protective agent, silymarin. The ACD/Chemsketch tool was used to generate 3D structures of ligands. A molecular file format converter tool has been used to convert the generated data to the protein Data Bank (PDB) and has been used for docking studies. The active site of the target protein was identified using Q-site finder tool. The energy values for docking interactions between the active site and the phytochemical constituents have been studied by using Flex X tool. Out of all inhibitors, silymarin, followed by 2-(2-methyleyclohexylidene)-hydrazine carboxamide possess the highest energy value indicating them as efficient inhibitors with the target proteins to treat hepatocellular carcinoma. The effective properties may be due to the presence of carbonyl and alholic OH groups present in the ligand molecules.*

**Keywords:** Molecular docking, Hepatocellular carcinoma, *Cucumis stativus* Linn., Hepatitis B X, Heme Oxygenase I, Silymarin.

**Received:** 3 February 2016/ **Revised:** 3 March 2016/ **Accepted:** 7 March 2016/ **Published:** 10 March 2016

## Contribution/ Originality

Cucumis sativus Linn. fruit has a number medicinal uses. The fruit is traditionally used by medical practitioners for curing liver disorder. The present study is a new original contribution. Six phytochemical constituents isolated and identified by GC-MS analysis by our group has been subjected to molecular docking studies for the first time with two novel hepato carcinoma receptors, Hepatitis B X and Hemo Oxygenase I whose 3D structures were retrieved from PDB database. Flex X docking program has been used to specify binding surface of the receptors and phytochemical constituents in SDF format. The present study gives an additional theoretical proof that the phytochemical constituents of the ethanol extract of Cucumis sativus can be used in the treatment of hepatocellular carcinoma which has already been proved pharmacologically by our group.

## 1. INTRODUCTION

Liver is a vital organ of paramount importance involved in the maintenance of metabolic functions and detoxification of the exogenous and endogenous challenges like xenobiotics, drugs, viral infections and chronic



† *Corresponding author*

alcoholism (Sharma and Sharma, 2010). It has great capacity to detoxicate toxic substances and synthesize useful principles (Shanani, 1999; Subramoniam and Pushpangadan, 1999). Liver damage is very common since liver has to detoxicate lot many toxic substances. Most of the hepatotoxic chemicals damage liver cells primarily by producing reactive species which form covalent bond with the lipids of the tissue. Production of the reactive species depletion manifests in tissue thiol depletion, lipid peroxidation, plasma membrane damage etc., culminating into severe hepatic injury (Gupta *et al*., 2009). About 20,000 deaths found every year due to liver disorders (Sharma and Kumar, 2010). Therefore, search for newer drugs, with minimum side effects obtained from Traditional medicines continues. *Cucumis sativus* Linn. has been widely used for the treatment of jaundice, burning sensation, haemostatic and other skin disorders (Aburjai and Natsheh, 2003). The juice is used in many beauty products (Katsambas and Lotti, 2003). Molecular docking methods are commonly used for predicting binding modes to proteins and energies of ligands (Bikadi and Hazai, 2009) which in turn predicts the strength of association or binding affinity between these molecules (Tripathi *et al*., 2011). Now a days applying computational methods for drug discovery and development are increasingly gaining popularity, implementation and appreciation (Shaikh *et al*., 2007). In the present investigation molecular docking studies of six phytochemical constituents isolated and identified from the ethanol extract of the fruits of *Cucumis sativus* Linn. have been carried out using the novel target receptors of hepato carcinoma, Hepatitis B X and Heme Oxygenase I by using the Flex X. Silymarin is used as the standard.

## 2. MATERIAL AND METHODS

### 2.1. Collection of Plant Materials

The fruits of *Cucumis sativus* Linn. was collected in the month of July from Alangulam, Tirunelveli District, Tamil Nadu and identified by Prof. P. Jayaraman, Plant Anatomy Research Centre, West Thambaram, Chennai-600 045, Tamil Nadu, India (Authentification Certificate Reg. No.: PARC/2013/2047).The voucher specimen (MSU/PHAR/HER–141) has been preserved in the Herbarium of the Department of Pharmaceutical Chemistry, Manonmaniam Sundaranar University, Tirunelveli-627 012, Tamil Nadu, India.

### 2.2. Instruments and Chromatographic Conditions

GC-MS analysis of the extract was carried out on a GC-MS Clarus 500 Perkin Elmer system comprising a AOC- 20i auto sampler and gas chromatograph interfaced to a mass spectrometer (GC-MS) instrument employing the following conditions: column Elite-1 fused silica capillary column (30 mm x 0.2 5mm ID x 1 µMdf, composed of 100 % Dimethyl poly siloxane), operating in electron impact mode at 70 eV; helium (99. 999 %) was used as carrier gas at a constant flow of 1ml/min and an injection volume of 0.5 µl was employed (split ratio of 10:1); injector temperature 250°C. The oven temperature was programmed from 110°C (isothermal for 2 min), with an increase of 10°C/min, to 200°C, then 5°C / min to 280°C, ending with a 9 min isothermal at 280°C. Mass spectra were taken at 70 eV; a scan interval of 0.5 seconds and fragments from 40 to 550 Da.

### 2.3. Identification of Phytochemical Constituents

Interpretation on mass spectra of GC-MS was conducted using the database of National Institute of Standards and Technology (NIST). The mass spectrum of the unknown component was compared with that of the known components stored in the NIST library. The name, molecular weight and structure of the SIX phytochemical constituents, 2,3-Dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one,5-(Hydroxylmethyl)-2-furancarboxaldehyde, 4-Hydroxy-3-methyl-2-butenyl-acetate, 2-(2-Methylcyclohexylidene)-hydrazinecarboxamide, n-Hexadecanoicacid, 1,2-Benzenedicarboxylicacid-diisooctylester isolated and identified from the ethanol extract of the fruits of *Cucumis sativus* Linn. were ascertained by GC-MS analysis (Sathyaprabha *et al*., 2011; Gopalakrishnan and Kalaiarasi, 2013) and are presented in Table.1.

### 2.4. Potential Targets and Binding Site

3D structures of hepatic cancer potential drug targets such as Hepatitis B X (3I7H), Heme Oxygenase I (1N3U) receptors were retrieved from PDB database (Berman *et al*., 2000). The active sites in these receptors were determined based on the ligands in the crystallized structures. The interactions and the affinities between the phytochemical constituents and receptor were predicted by using Flex X docking program (Rarey *et al*., 1996).

### 2.5. Ligand Generation

2D structures of phytochemical constituents isolated and identified from the ethanol extract of the fruits of *Cucumis sativus* were drawn in ACD-Chemsketch (ACD, 2006) and their SMILES notations were obtained. 3D structures were obtained and converted into SDF files by using 'Online SMILES convertor and Structure file generator' server (Weininger, 1988).

### 2.6. Flexible Docking

The binding affinities of the phytochemical constituents were predicted by docking the phytochemical constituents within the binding sites of hepatic cancer potential drug targets by using Flex X with the following parameters i) default general docking information ii) base placement using triangle matching, iii) scoring of full score contribution and threshold of 0,30 and No score contribution and threshold of 0,70. iv) Chemical parameters of clash handling values for protein ligand clashes with maximum allowed overlap volume of 2.9 A and intra-ligand clashes with clash factor of 0.6 and considering the hydrogen in internal clash tests. v) Default docking details values of 200 for both the maximum number of solutions per iteration and maximum number of solutions per fragmentation.

### 2.7. Prediction of Ligand- Receptor Interactions

The interactions between the SIX phytochemical constituents isolated and identified from the ethanol extract of the fruits of *Cucumis sativus*, and the two novel receptors as docked complexes were analyzed by the pose-view of Lead IT (Stierand *et al*., 2006).

## 3. RESULTS AND DISCUSSION

Globally, the hepatocellular carcinoma (HCC) is the fifth and third most common cancer that leads to the cancer-related death. The prognosis of HCC is very poor without specific treatments that averaged the median survival of patients to 1-2 months with advanced tumors. HCC more often arises on virus-induced liver cirrhosis, thus outlining a model of disease progression from chronic inflammation to cancer and allowing design of new strategies targeting key targets at each step of the disease. In the present study two novel hepato carcinomo receptors, Hepatitis B X, and Heme Oxygenase I were selected as a potential drug targets of HCC. 3D structures of Hepatitis B X, and Heme Oxygenase I were determined and the molecular docking studies of the six phytochemical constituents isolated and identified from the ethanol extract of the fruits of *Cucumis sativus* have been performed. The receptors, Hepatitis B X and Heme Oxygenase I were considered as the potential drug targets of HCC and their 3D structures were retrieved from Protein Databank (Figure. 1) and their binding sites were determined. The Docking program, from Lead IT (Flex X) was used to specify binding surface of the receptors and the phytochemical constituents in SDF format. The docking was carried out with the radius of 6.5 $A^0$ at the site of docking.

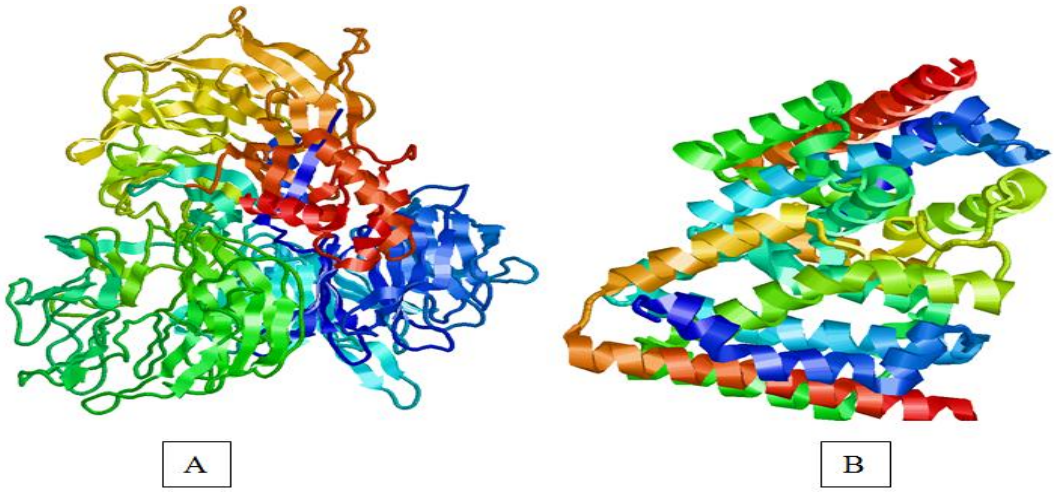
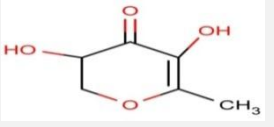
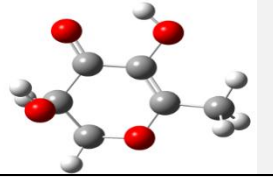
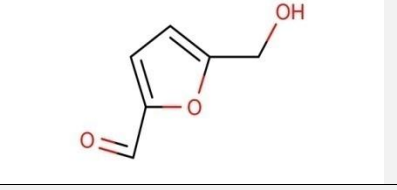
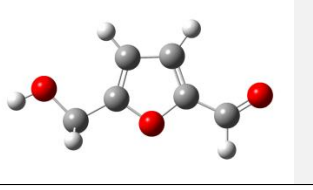
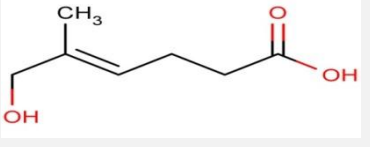
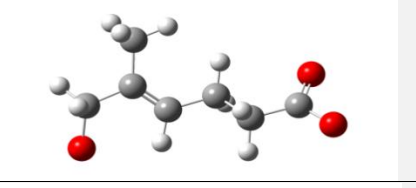
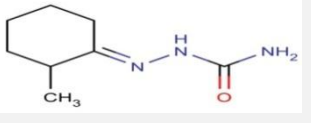
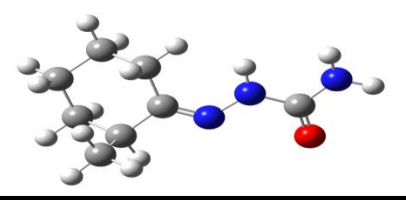


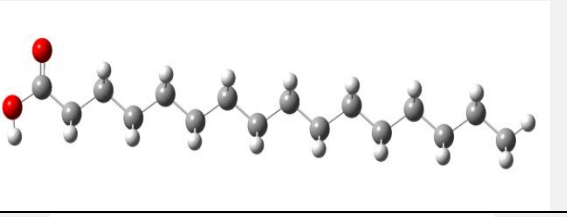
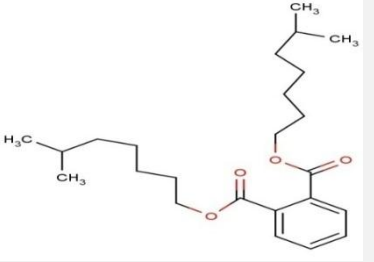
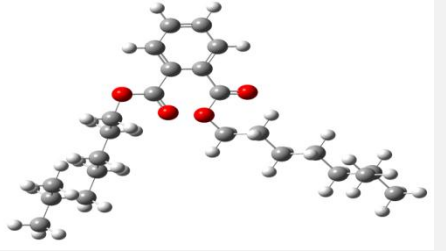
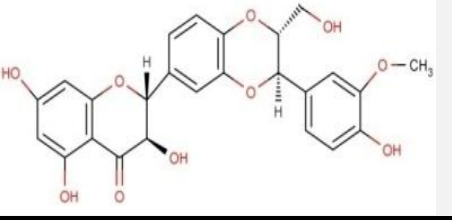
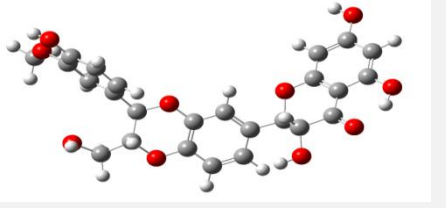

**Fig-1.** 3D Structures of A) Hepatitis B X, B) Heme Oxygenase I.

**Source:** Protein Data Bank Database

2D and 3D structures of the six phytochemical constituents isolated and identified from the ethanol extract of the fruits of *Cucumis sativus* Linn.are presented in Table. 1. The docking interactions between the binding site amino acids of Hepatitis B X, and Heme Oxygenase I and the 7 ligand molecules are presented in Table. 2. Silymarin (Std) is found to be the best docking ligand with Hepatitis B X (Figure. 2) and Heme Oxygenase I (Figure 3), followed by 2-(2-Methylcyclohexylidene)-hydrazinecarboxamide. The results of hydrogen bonding and hydrophobic interactions of ligand molecules with Hepatitis B X and Heme Oxygenase I are presented in Table. 3.

**Table-1.** 2D and 3D Structures of the six phytochemical constituents isolated and identified from the ethanol extract of the fruits of *Cucumis sativus* Linn. and Silymarin (Std).

| S. No. | Ligands | 2D structure | 3D structure |
|---|---|---|---|
| 1 | **2,3-Dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one** |  |  |
| 2 | **5-(Hydroxymethyl)- 2-furancarboxaldehyde** |  |  |
| 3 | **4-Hydroxy-3-methyl-2-butenyl-acetate** |  |  |
| 4 | **2-(2-Methylcyclohexylidene)-hydrazinecarboxamide** |  |  |

| | | | |
|---|---|---|---|
| 5 | **n-Hexadecanoic acid** |  |  |
| 6 | **1,2-Benzenedicarboxylic acid-diisooctyl ester** |  |  |
| 7 | **Silymarin (Std)** |  |  |

**Source:** 2D Structure-ACD-Chemsketch; 3D Structure-Online SMILES Convertor and Structure file Generator Server

**Table-2.** Docking score of the six phytochemical constituents isolated and identified from the ethanol extract of the fruits of *Cucumis sativus* Linn .and Silymarin (Std) with Hepatitis B X and Heme oxygenase I.

| S. No. | Ligand | Docking score of | |
|---|---|---|---|
| | | Hepatitis B X kJ/mol | Heme Oxygenase I kJ/mol |
| 1 | 2,3-Dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one | -9.9908 | -13.9858 |
| 2 | 5-(Hydroxymethyl)-2-furancarboxaldehyde | -12.1585 | -13.7395 |
| 3 | 4-Hydroxy-3-methyl-2-butenyl- acetate | -13.1061 | -13.7954 |
| 4 | 2-(2-Methylcyclohexylidene)- hydrazinecarboxamide | -16.3483 | -17.0479 |
| 5 | n-Hexadecanoic acid | -1.7388 | -3.5345 |
| 6 | 1,2-Benzenedicarboxylic acid-diisooctyl ester | -1.5955 | -4.2210 |
| 7 | Silymarin (Std) | -17.3005 | -18.9744 |

**Source:** Flex X docking Program

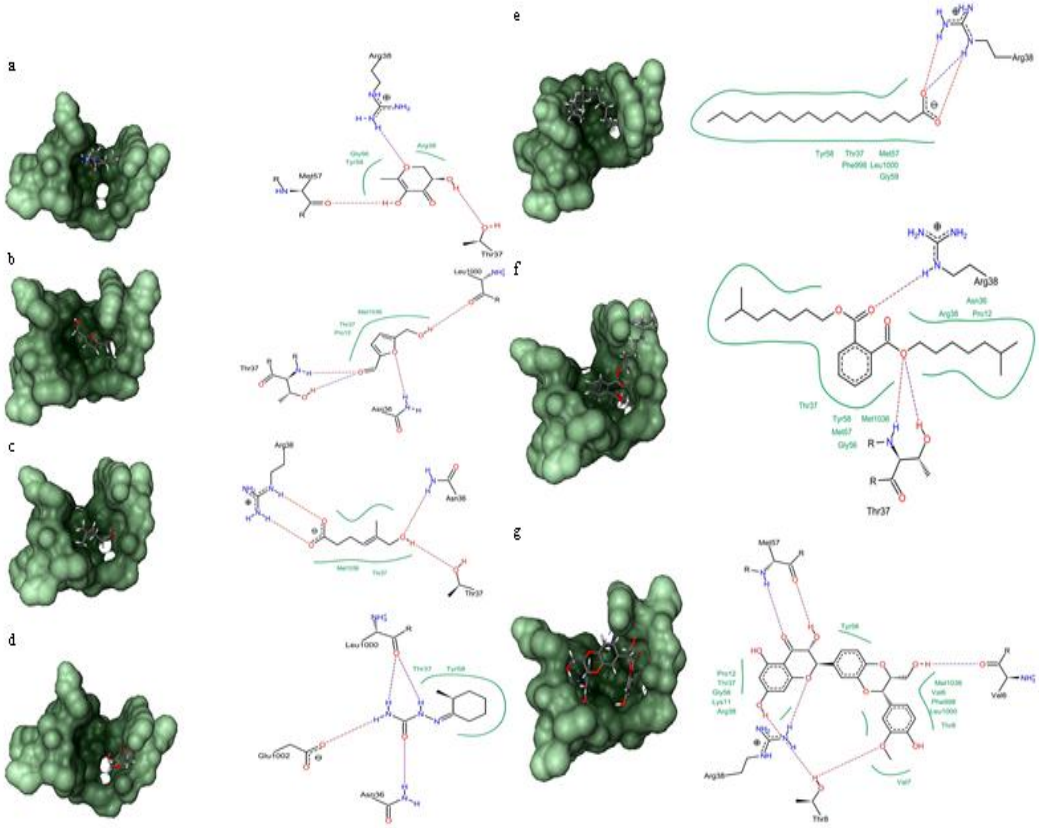



**Fig-2.** Hydrogen bonding and hydrophobic interactions of phytochemical constituents isolated and identified from the ethanol extract of the fruits of *Cucumis sativus* Linn.and Silymarin (Std) with Hepatitis B X.
a) 2,3-Dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one b) 5-(Hydroxymethyl)- 2-furancarboxaldehyde c)4-Hydroxy-3-methyl-2-butenyl-acetate d) 2-(2-Methylcyclohexylidene)-hydrazinecarboxamide e) n-Hexadecanoic acid f) 1,2-Benzenedicarboxylic acid-diisooctyl ester g) Silymarin (Std).

**Source:** 3D Structure-Online SMILES Convertor and Structure file Generator Server; Flex X docking Program

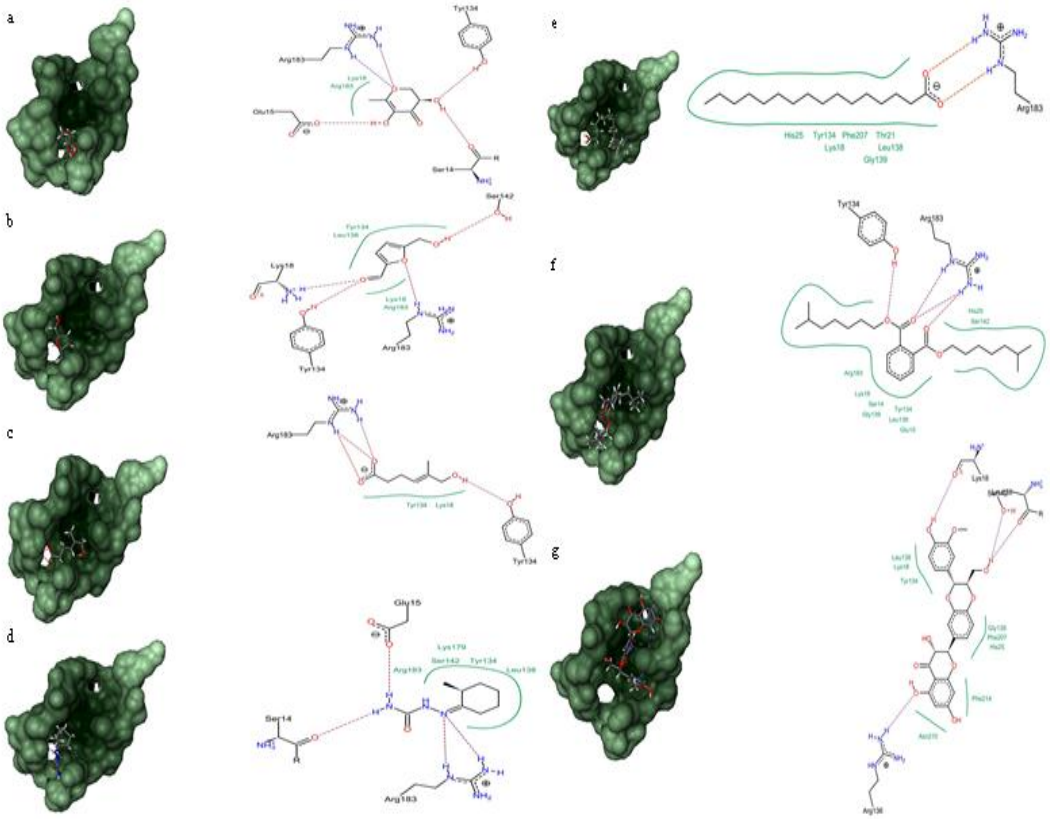


**Fig-3.**Hydrogen bonding and hydrophobic interactions of phytochemical constituents isolated and identified from the ethanol extract of the fruits of *Cucumis sativus* Linn. and Silymarin (Std) with Heme Oxygenase I.
a) 2,3-Dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one b) 5-(Hydroxymethyl)- 2-furancarboxaldehyde c)4-Hydroxy-3-methyl-2-butenyl-acetate d) 2-(2-Methylcyclohexylidene)-hydrazinecarboxamidee) n-Hexadecanoic acid f) 1,2-Benzenedicarboxylic acid-diisooctyl ester g) Silymarin (Std).

**Source:** 3D Structure-Online SMILES Convertor and Structure file Generator Server; Flex X docking Program

Hepatitis B X plays a vital role in hepatitis infection and a need for Hepatitis B X targeting drugs rendered it as a target for our study (Manjunatha *et al*., 2010). In the fruits of *Cucumis sativus* Linn., the highest docking interactions score (-17.3005 kJ/mol) was observed for silymarin (Std) with the Hepatitis B X receptor. The interactions are favored by Met 57, Val 6, Arg 38 and Thr 8 by hydrogen bond formation and hydrophobic formations by means of Pro 12, Thr 37, Gly 56, Lys 11, Arg 38, Val 7, Met 1036, Val 6, Phe 998, Leu 1000 and Thr 8. The binding of remaining phytochemical constituents which exhibited the docking score ranging from-17.3005 kJ/mol to -1.5955 kJ/mol. 2-(2-Methylcyclohexylidene)-hydrazinecarboxamide exhibited the docking score of -16.3483 kJ/mol. The interactions is favored by Glu 1002, Asn 36 and Leu 1000 by hydrogen bond formation and hydrophobic formations by means of Thr 37and Tyr 58. It is observed that in this case also the NH group of the amino acid and the carbonyl group present in the phytochemical constituents favor the hydrogen bond interactions. The findings also envisage that during the design of novel hepatoprotective compounds, the conserved amino acids have to be considered for enhancing the hepatoprotective activity of the phytochemical constituents against Hepatitis B X.

**Table-3.** Hydrogen bonding and hydrophobic interactions of the six phytochemical constituents isolated and identified from the ethanol extract of the fruits of *Cucumis sativus* Linn. and Silymarin (Std) with Hepatitis B X and Heme Oxygenase I.

| S. No. | Ligand | Hepatitis B X | | Heme Oxygenase I | |
|---|---|---|---|---|---|
| | | Hydrogen bonding interactions | Non bonded interactions | Hydrogen bonding interactions | Non bonded interactions |
| 1 | 2,3-Dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one | Met 57, Arg 38, Thr 37 | Gly 56, Tyr 58, Arg 38 | Glu 15, Arg 183, Tyr 134, Ser 14 | Arg 183, Lys 18 |
| 2 | 5-(Hydroxymethyl)-2-furancarboxaldehyde | Thr 37, Asn 36, Leu 1000 | Pro 12, Thr 37, Met 1036 | Lys 18, Tyr 134, Arg 183, Ser 142 | Tyr 134, Leu 138, Lys 18, Arg 183 |
| 3 | 4-Hydroxy-3-methyl-2-butenyl- acetate | Arg 38, Asn 36, Thr 37 | Met 1036, Thr 37 | Arg 183, Tyr 134 | Tyr 134, Lys 18 |
| 4 | 2-(2-Methylcyclohexylidene)-hydrazinecarboxamide | Glu 1002, Asn 36, Leu 1000 | Thr 37, Tyr 58 | Ser 14, Arg 183, Glu 15 | Arg 183, Ser 142, Lys 179, Tyr 134, Leu 138 |
| 5 | n-Hexadecanoic acid | Arg 38 | Tyr 58, Thr 37, Phe 998, Met 57, Leu 1000, Gly 59 | Arg 183 | His 25, Tyr 134, Lys 18, Phe 207, Thr 21, Leu 138, Gly 139 |
| 6 | 1,2-Benzenedicarboxylic acid-diisooctyl ester | Arg 38, Thr 37 | Thr 37, Tyr 58, Met 57, Gly 56, Met 1036, Arg 38, Asn 36, Pro 12 | Tyr 134, Arg 183 | Arg 183, Lys 18, Ser 14, Gly 139, Tyr 134, Leu 138, Glu 15, His 25, Ser 142 |
| 7 | Silymarin (Std) | Met 57, Val 6, Arg 38, Thr 8 | Pro 12, Thr 37, Gly 56, Lys 11, Arg 38, Tyr 58, Val 7, Met 1036, Val 6, Phe 998, Leu 1000, Thr 8 | Arg 136, Lys 18, Ser 142, Leu 118 | Asn 210, Phe 214, Gly 139, Phe 207, His 25, Leu 138, Lys 18, Tyr 134 |

**Source:** Flex X docking Program

The highest docking interactions score (-18.9744 kJ/mol) was observed for silymarin with the Heme Oxygenase I receptor also. The best docking interactions of silymarin is favored by the formation of hydrogen bond with Lys 18, Ser 142, Leu 188 and Arg 136. The hydrophobic interactions are contributed by Leu 138, Lys 18, Tyr 134, Gly 139, Phe 207, His 25, Phe 214 and Asn 210. The binding of remaining phytochemical constituents which exhibited the docking score ranging from -18.9744 kJ/mol to -3.5345 kJ/mol. 2-(2-Methylcyclohexylidene)-hydrazinecarboxamide exhibited the docking score of -17.0479 kJ/mol. The interactions are favored by Ser 14, Arg 183 and Glu 15 by hydrogen bond formation and hydrophobic formations by means of Arg 183, Ser 142, Lys 179, Tyr 134 and Leu 138. It is observed that in this case also the NH group of the amino acid and the carbonyl group present in the phytochemical constituents favor the hydrogen bond interactions. The findings envisage that during the design of novel hepatoprotective compounds, the conserved amino acids have to be considered for enhancing the hepatoprotective activity of the phytochemical constituents against Heme Oxygenase I .Heme Oxygenase I products, the induction of this enzyme or its catalytic activity by the Phytochemical constituents may represent an effective strategy to intervene in liver carcinogenesis and other hepatic disorders (Ebenezer and Young-Joon, 2006).

## 4. CONCLUSION

Molecular Docking continues to hold great promise in the field of computer based drug design, which screens small molecules by orienting and scoring them in the binding site of a protein. The present study indicates that the phytochemical constituents from the ethanol extract of the fruits of *Cucumis sativus* Linn. can be used in the treatment of hepatocellular carcinomo, which shows a strong binding affinity towards Hepatitis B X and Heme Oxygenase I. The analysis of the docking result allowed us to know the phytochemical constituents of *Cucumis sativus* Linn. as an antagonist to hepatocellular carcinomo receptor.

Funding: This study received no specific financial support.

Competing Interests: The authors declare that they have no conflict of interests.

Contributors/Acknowledgement: All authors contributed equally to the conception and design of the study.

## REFERENCES

Aburjai, T. and F.M. Natsheh, 2003. Plants used in cosmetics. Phytotherapy Research, 17(9): 987-1000.

ACD, 2006. ACD/chemsketch freeware, version 11 advanced chemistry development, Inc. Toronto, ON, Canada. Available from www.acdlabs.com.

Berman, H.M., J. Westbrook and Z. Feng, 2000. The protein data bank. Nucl Acids Res, 28(1): 235–242.

Bikadi, Z. and E. Hazai, 2009. Application of the PM6 semi-empirical method to modeling proteins enhances docking accuracy of autodock. J. Cheminf, 1: 1-15.

Ebenezer, O.F. and S. Young-Joon, 2006. Journal of Biochemistry and Molecular Biology, 39(5): 479-491.

Gopalakrishnan, S. and T. Kalaiarasi, 2013. Determination of biologically active constituents of the fruits of cucumis sativus Linn. Using GC-MS analysis. International Journal of Biological and Pharmaceutical Research, 4(7): 523-527.

Gupta, A.K., P. Ganguly, U.K. Majumder and S. Ghosal, 2009. Hepatoprotective & antioxidant effect & stereoidal saponins of solanum of solanum xanthocarpum & solanum nigrum in paracetomol induce hepatotoxicity in rats. Pharmacologyonline, 1(27): 757-768.

Katsambas, A.D. and T.M. Lotti, 2003. European handbook of dermatological treatment. 2nd Edn., Berlin, Germany: Springer Verlag. pp: 336-341.

Manjunatha, B.K., R.R. Amit and P. Priyanka, 2010. Lead finding from pterocarpus santalinus with hepatoprotective potentials through in silico methods. International Journal of Pharma Sciences and Research, 1(7): 265-270.

Rarey, M., B. Kramer and T. Lengauer, 1996. A fast flexible docking method using an incremental construction algorithm. J Mol Biol, 261(3): 470–489.

Sathyaprabha, G., S. Kumaravel and A. Panneerselvam, 2011. Bioactive compounds identification of pleurotus platypus and pleurotus eous by GC-MS. Adv. Appl. Sci. Res, 2(6): 51-54.

Shaikh, S.A., T. Jain, G. Sandhu, N. Latha and B. Jayaram, 2007. From drug target to leads- sketching a physicochemical pathway for lead molecule design in silico. Curr Pharm Des, 13(34): 3454-3470.

Shanani, S., 1999. Evaluation of hepatoprotective efficacy of APCL-a polyherbal formulation in vivo in rats. Indian Drugs, 38: 628-631.

Sharma, B. and S.U. Kumar, 2010. Hepatoprotective activity of some indigenous plants. International Journal of Pharm Tech Research, 2(1): 568-572.

Sharma, B. and U.K. Sharma, 2010. Hepatoprotective activity of some indigenous plants. Int J Pharm Tech Res, 2(1): 568-572.

Stierand, K., P. Maab and M. Rarey, 2006. Molecular complexes at a glance: Automated generation of two-dimensional complex diagrams. Bioinformatics, 22(14): 1710–1716.

Subramoniam, A. and P. Pushpangadan, 1999. Development of phytomedicine for liver diseases. Indian J. Pharmacol, 31(3): 166-175.

Tripathi, L., P. Kumar, J. Haneef and R. Singh, 2011. Molecular docking softwares: An overview. Pharma Rev, 7: 69-74.

Weininger, D., 1988. Smiles, a chemical language and information system. Introduction to methodology and encoding rules. J Chem. Inf. Comput. Sci, 28(1): 31–36.