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Research Article

Molecular docking studies of phytochemical constituents of the fruits of *Cucumis trigonus roxb.* against hepatocarcinomo receptors

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ABSTRACT

Hepatocarcinomo continues to be a worldwide killer, despite the enormous amount of research and rapid developments seen during the past decade. Since it is commonly believed that many are preventable, there is urgent need to identify natural medicines as effective hepatoprotective agents. Natural products identified and isolated from plants have played an important role in discovery of drugs against liver diseases. In silico docking techniques are being used to investigate the complementarily at the molecular level of a ligand and a protein target. In the present study, nine ligands which have been isolated and identified from the ethanolic extract of the fruits of Cucumis trigonus Roxb. and the standard hepatoprotective agent silymarin, are docked with two novel hepatocacrcinomo receptors, Hepatitis B X and Heme Oxygenase I. Out of the nine phytochemical constituents isolated and identified from the ethanolic extract of the fruits of Cucumis trigonus, demeclocycline ligand revealed the best fitness score (-17.5103 kJ/mol) compared with the standard drug, silymarin. This suggests that demeclocycline could be an effective potential inhibitor against Hepatitis B X receptor and can be evaluated as hepatoprotective drug molecule using molecular docking studies. These effective properties may be due to the presence of carbonyl and alcoholic-OH functional groups present in the ligand molecules.

Key words: Molecular docking, *Cucumis trigonus Roxb*, Hepatocarcinoma, Hepatitis B X, Heme Oxygenase I, Demeclocycline, Silymarin.

INTRODUCTION

The liver plays an astonishing array of vital functions in the maintenance, performance and regulating homeostasis of the body. It has great capacity to detoxicate toxic substances and synthesize useful principles1,2. Liver diseases remain one of the major threats to public health and are a worldwide problem3. They are mainly caused by chemicals like acetaminophen, excess consumption of alcohol, infections and autoimmune disorders. Most of the hepatotoxic chemicals damage liver cells mainly by inducing lipid peroxidation and other oxidative damages4-6. Unfortunately, conventional or synthetic drugs used in the treatment of liver disease are inadequate and sometimes cause serious side effects. Therefore, it is necessary to search for alternative drugs for the treatment of liver disease with more efficacy and safety and to improve, augment, or replace currently used drugs7,8. Therefore, search for newer drugs with minimum side effects obtained from traditional medicines continues. Scientific studies available on medicinal plants indicate that promising phytochemicals can be developed for many health problems9. Computational Biology and Bioinformatics have the potential not only of speeding up the drug discovery process but reduces the costs, and also of changes the way drugs are designed. One such method is the docking of the drug molecule with the target receptor10. Molecular docking studies are used to determine the interaction of two molecules and to find the best orientation of ligand which would form a complex with overall minimum energy. The small molecule, known as ligand usually fits within protein's cavity which is predicted by the search algorithm. These protein cavities become active when come in contact with any external compounds and are thus called as active sites. In the present investigation molecular docking studies of the nine phytochemical constituents isolated and identified from the ethanolic extract of the fruits of Cucumis trigonus Roxb. have been carried out using two novel target receptors of

hepatocarcinoma, Hepatitis B X and Heme Oxygenase I by using the Flex X. Silymarin is used as the standard.

MATERIALS AND METHODS

Collection of Plant Materials:

The fruits of *Cucumis trigonus* were collected in the month of March 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/2013/2048). The voucher specimen (MSU/PHAR/HER–140) has been preserved in the Herbarium of the Department of Pharmaceutical Chemistry, Manonmaniam Sundaranar University, Tirunelveli -627 012, Tamil Nadu, India.

Instruments and Chromatographic Conditions:

GC-MS analysis of the extracts was carried out on a GC-MS Clarus 500 Perkin Elmer system comprising a AOC-20i autosampler 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.

Identification of Photochemical 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 nine phytochemical constituents demeclocycline, glycodeoxycholic acid, 3α,7α,12α-trihydroxycoprostanic acid, chlortetracycline, azafrin methyl ester, giganteumgenin N, phorbol 12,13-dihexanoate, astaxanthin, tetrahydrospirilloxanthinisolated and identified from the ethanol extract of the fruits of *Cucumis trigonus* Roxb. were ascertained by GC-MS analysis[11,12] and are presented in Table.1.

Potential Targets and Binding Site:

The 3D structures of hepatic cancer potential drug targets such as Hepatitis B X (317H), Heme Oxygenase I (1N3U) receptors were retrieved from PDB database[13]. 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[14].

Ligand Generation:

The 2D structures of phytochemical constituents from the ethanolic extract of the fruits of *Cucumis trigonus* were drawn in ACD-Chemsketch[15] and their SMILES notations were obtained. The 3D structures were obtained and converted into SDF files by using 'Online SMILES convertor and Structure file generator' server[16].

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.

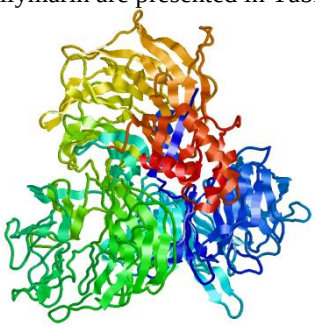
Prediction of Ligand- Receptor Interactions:

The interactions between the nine phytochemical constituents isolated and identified from the ethanolic extract of the fruits of *Cucumis trigonus*, and the two novel receptors as docked complexes were analyzed by the pose-view of Lead IT[17].

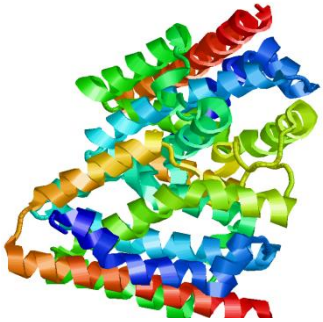
RESULTS AND DISCUSSION

Diverse homeostatic mechanisms are affected if liver function is impaired, with potentially serious consequences. About 20, 000 deaths occur every year due to liver diseases. Hepatocellular carcinoma (HCC) is one of the ten most common tumors in the world with over 2, 50,000 new cases each year. 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. Thus in the present study two novel receptors, Hepatitis B X and Heme Oxygenase I were selected as a potential drug targets of Hepatocellular carcinoma. 3D structures of Hepatitis B X and Heme Oxygenase I were determined and the molecular docking studies of the nine photochemical constituents isolated and identified from the ethanolic extract of the fruits of *Cucumis trigonus* 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**.** 2D and 3D structures of the nine phytochemical constituents isolated and identified from the

ethanol extract of the fruits of *Cucumis trigonus* Roxb.and the standard, Silymarin are presented in Table. 1.



(a)

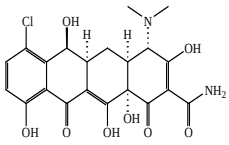
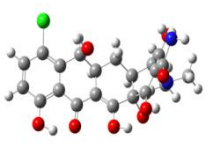
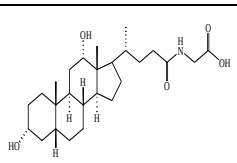
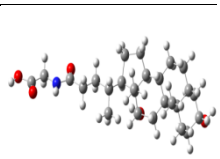
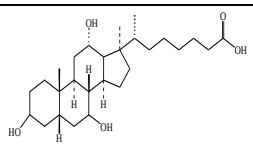
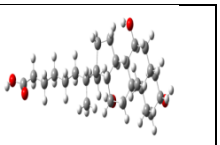
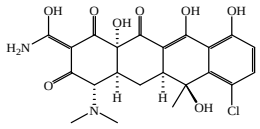
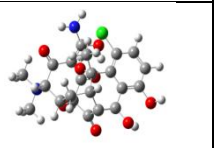
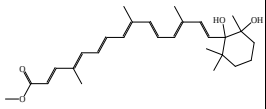
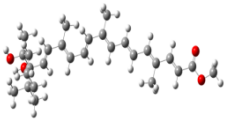
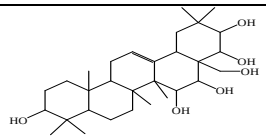
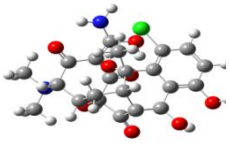
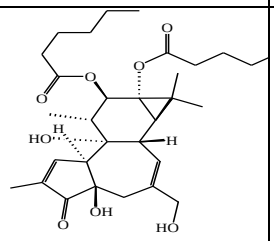
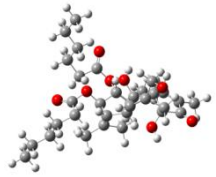
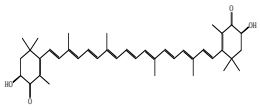
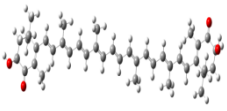
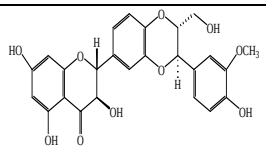
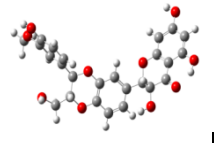


(b)

Figure 1 : 3D Structures of a) Hepatitis B X, b) Heme Oxygenase I

The docking interactions between the binding site amino acids of Hepatitis B X and Heme Oxygenase I and the 10 ligand molecules are presented in Table. 2. Demeclocycline is found to be a best docking ligand compared to Silymarin with Hepatitis B X (Figure. 2). On the other hand Silymarin (Std) is found to be the best docking ligand with Heme Oxygenase I followed by Glycodeoxycholic acid (Figure. 3). 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 nine phytochemical constituents isolated and identified from the ethanol extract of the fruits of *Cucumis trigonus* Roxb. and Silymarin (Std)

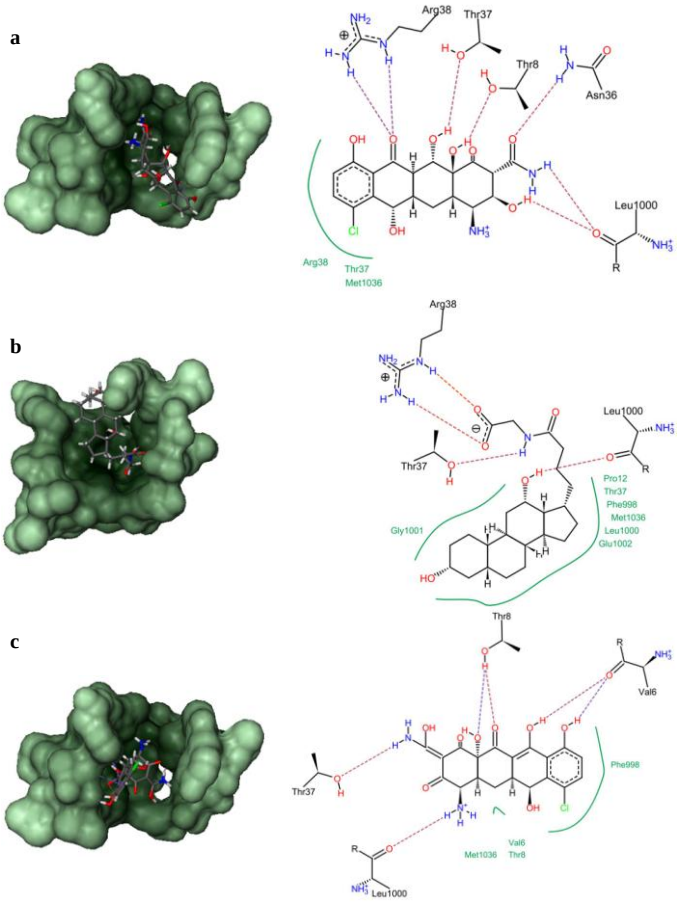
S. No.	Ligands	2D structure	3D structure
1	Demeclocycline		
2	Glycodeoxycholic acid		
3	3α,7α,12α - trihydroxycoprostanic acid		
4	Chlortetracycline		
5	Azafrin methyl ester		
6	Giganteumgenin N		
7	Phorbol 12,13-dihexanoate		
8	Astaxanthin		
9	Tetrahydrospirilloxanthin		
10	Silymarin (Std)		

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[18]. The highest docking interactions score was observed for demeclocycline (-17.5103 kJ/mol) with the Hepatitis B X receptor. The compounds, 3α,7α,12α-trihydroxycoprostanic acid, astaxanthin, and tetrahydrospirilloxanthin do not exhibit any binding within Hepatitis B X active site. The best docking interactions of

demeclocycline is favored by the formation of hydrogen bond with Arg 38, Thr 37, Thr 8, Asn 36 and Leu 1000. The hydrophobic interactions are contributed by Arg 38, Thr 37 and Met 1036. The binding of remaining phytochemical constituents which exhibited the docking score ranging from -17.5103 kJ/mol to -1.3674 kJ/mol.

Table 2 :Docking score of the nine phytochemical constituents isolated and identified from the ethanol extract of the fruits of *Cucumis trigonus* Roxb. 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	Demeclocycline	-17.5103	-14.0168
2	Glycodeoxycholic acid	-15.4232	-16.2795
3	3α,7α,12α –Trihydroxy coprostanic acid	-	-
4	Chlortetracycline	-14.9852	-13.2065
5	Azafrin methyl ester	-3.5081	-11.6456
6	Giganteumgenin N	-5.1845	-0.8337
7	Phorbol 12,13-dihexanoate	-1.3674	-0.6923
8	Astaxanthin	-	-13.2514
9	Tetrahydrospirilloxanthin	-	-2.9851
10	Silymarin (Std)	-17.3005	-18.9744



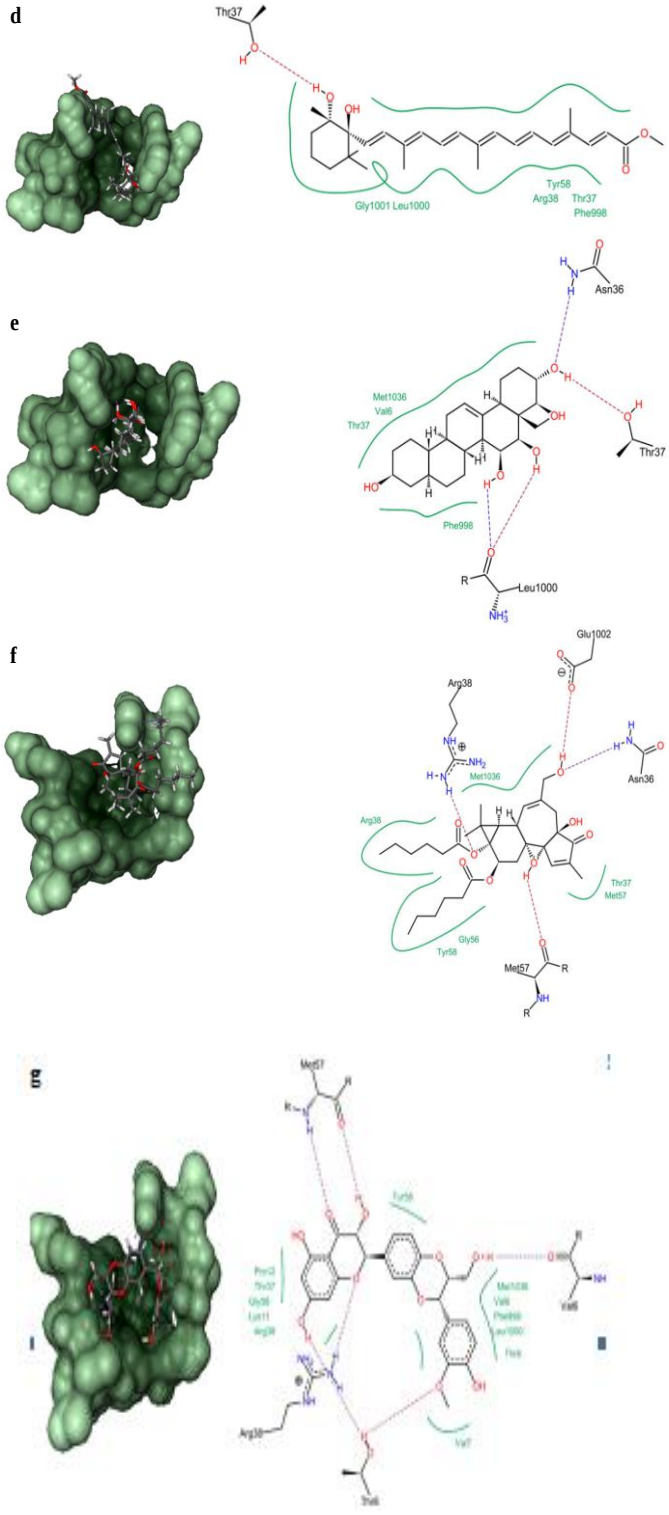


Figure. 2: Hydrogen bonding and hydrophobic interactions of phyto chemical constituents isolated and identified from the ethanol extract of the fruits of *Cucumis trigonus* Roxb. And Silymarin (Std.) with Hepatitis B X. a) Demeclocycline b) Glycodeoxycholic acid c) Chlortetracycline d) Azafrin methyl ester e) Giganteumgenin N f) Phorbol 12,13-dihexanoate g) Silymarin (Std).

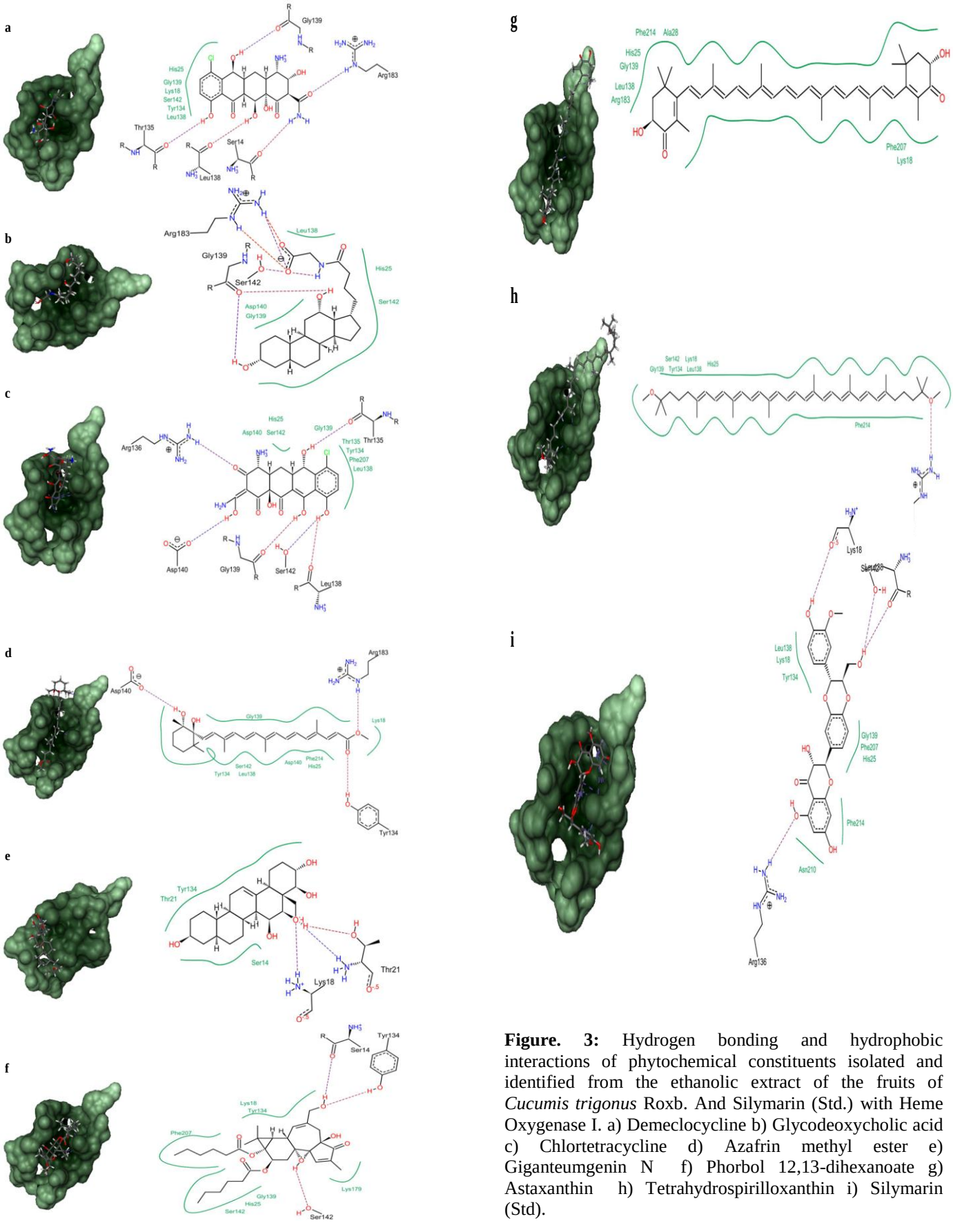


Figure. 3: Hydrogen bonding and hydrophobic interactions of phytochemical constituents isolated and identified from the ethanolic extract of the fruits of *Cucumis trigonus* Roxb. And Silymarin (Std.) with Heme Oxygenase I. a) Demeclocycline b) Glycodeoxycholic acid c) Chlortetracycline d) Azafrin methyl ester e) Giganteumgenin N f) Phorbol 12,13-dihexanoate g) Astaxanthin h) Tetrahydrospirilloxanthin i) Silymarin (Std).

The well known hepatic cancer drug, silymarin which exhibited the dock score of -17.3005 kJ/mol. 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. Interestingly it is observed that the amino acid, Arginine (Arg 38) is found to be conserved in all the cases of docking interactions of the phytochemical constituents. The docking study implies that the conserved amino acids, Arg 38 and Thr 8 in the active site of Hepatitis B X receptor plays a crucial role by hydrogen bond interactions and the amino acids, Arg 38, Thr 37 and Met 1036. for the non bonded interactions. It is observed that the NH group of the amino acid and the carbonyl group present in the phyto chemical constituents favour the hydrogen bond interactions. The findings envisage that during the design of novel hepatoprotective compounds, the conserved amino acids, arginine (R), threonine (T), and methionine (M) are to be considered for enhancing the hepatoprotective activity of the phyto chemical constituents against Hepatitis B X. The highest docking interactions score (-18.9744 kJ/mol) was observed for Silymarin with the Heme Oxygenase I receptor. 3α,7α,12α-trihydroxycoprostanic acid does not exhibit any binding interaction within the Heme Oxygenase I active site. 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 -0.6923 kJ/mol followed by Glycodeoxycholic acid exhibited the docking score of -16.2795 kJ/mol. The interactions are favored by Arg 183, Gly 139 and Ser 142 by hydrogen bond formation and hydrophobic formations by means of Leu 138, His 25, Ser 142, Asp 140 and Gly 139. It is observed that the NH group of the amino acid and the carbonyl group present in the phytochemical constituents favour 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 products, the induction of this enzyme or its catalytic activity by either natural or synthetic compounds may represent an effective strategy to intervene in liver carcinogenesis and other hepatic disorders[19].

Table 3: Hydrogen bonding and hydrophobic interactions of the nine phytochemical constituents isolated and identified from the ethanolic extract of the fruits of *Cucumis trigonus* Roxb. 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	Demeclocycline	Arg 38, Thr 37, Thr 8, Asn 36, Leu 1000	Arg 38, Thr 37, Met 1036	Thr 135, Leu 138, Ser 14, Gly 139, Arg 183	His 25, Gly 139, Lys 18, Ser 142, Tyr 134, Leu 138
2	Glycodeoxycholic acid	Arg 38, Thr 37, Leu 1000	Gly 1001, Pro 12, Thr 37, Phe 998, Met 1036, Leu 1000, Glu 1002	Arg 183, Gly 139, Ser 142	Leu 138, His 25, Ser 142, Asp 140, Gly 139
3	3α,7α,12α – trihydroxycoprostanic acid	-	-	-	-
4	Chlortetracycline	Thr 37, Leu 1000, Thr 8, Val 6	Met 1036, Val 6, Thr 8, Phe 998	Arg 136, Asp 140, Gly 139, Ser 142, Leu 138, Thr 135	Asp 140, His 25, Ser 142, Gly 139, Thr 135, Tyr 134, Phe 207, Leu 138
5	Azafrin methyl ester	Thr 337	Gly 1001, Leu 1000, Tyr 58, Arg 38, Thr 37, Phe 998	Asp 140, Arg 183, Tyr 134	Tyr 134, Ser 142, Leu 138, Asp 140, Phe 214, His 25, Gly 139, Lys 18
6	Giganteumgenin N	Asn 36, Thr 37, Leu 1000	Phe 998, Thr 37, Val 6, Met 1036	Lys 18, Thr 21	Thr 21, Tyr 134, Ser 14
7	Phorbol 12,13-dihexanoate	Arg 38, Glu 1002, Asn 36, Met 57	Arg 38, Met 1036, Thr 37, Met 57, Gly 56, Tyr 58	Ser 142, Ser 14, Tyr 134	Phe 207, Lys 18, Tyr 134, Ser 142, His 25, Gly 139, Lys 179
8	Astaxanthin	Thr 37	Gly 1001, Leu 1000, Tyr 58, Arg 38, Thr 37, Phe 998	-	Arg 183, Leu 138, Gly 139, His 25, Phe 214, Ala 28, Phe 207, Lys 18
9	Tetrahydrospirilloxanthin	-	-	Arg 183	Gly 139, Ser 142, Tyr 134, Lys 18, Leu 138, His 25, Phe 214
10	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	Lys 18, Ser 142, Leu 118 Arg 136,	Asn 210, Phe 214, Gly 139, Phe 207, His 25, Leu 138, Lys 18, Tyr 134

CONCLUSION

The development of novel compounds with biological activity is an urgent need. The molecular docking study revealed that the binding orientations of the phytochemical constituents from the ethanolic extract of the fruits of *Cucumis trigonus* Roxb. with the active site of the target proteins, Hepatitis B X and Heme Oxygenase I. The present study confirms that phytochemical constituents with interesting biological properties and structural diversity may serve as valuable lead drug candidates for the treatment of liver diseases. This study may provide an insight for exploitation of drugs from phytochemical constituents against hepatocarcinomo receptors of different types in close proximity to future.

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