Journal of Pharma Research
Available online through
www.jprinfo.com

Research Article
ISSN: 2319-5622

Evaluation of Phytochemical Constituents of *Cucumis trigonus* Roxb. for their Hepatoprotective Activity by Molecular Docking studies

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Received on: 06-05-2015; Revised and Accepted on: 20-05-2015

ABSTRACT

Docking is a term used for computational scheme that attempts to find the best matching between two molecules: a receptor and a ligand. Computer-Aided Drug Design (CADD) is a specialized discipline that uses computational methods to stimulate drug-protein interaction. Nine phytochemical constituents isolated and identified from the ethanolic extract of the fruits of Cucumis trigonus Roxb. by GC-MS technique have been subjected to molecular docking studies using a novel hepatocarcinoma receptor, Alpha-antitrypsin and a commonly used hepatoprotective receptor, CytochromeP^{450}. Silymarin is used as a standard hepatoprotective agent. The ACD/Chemsketch tool was used to generate 3D structures of the ligands. A molecular file format converter tool has been used to convert the generated data to the PDB format (Protein Data Bank) and has been used for docking studies. The active site of the target protein was identified using a Q-site finder tool. The ability of the ligands to bind with the active sites of Alpha-antitrypsin and CytochromeP^{450} have been studied using the Flex X tool. Out of the nine phytochemical constituents isolated and identified from the ethanolic extract of the fruits of Cucumis trigonus, Glycodeoxycholic acid was found to be the effective drug, selected on the basis of its docking score with the target proteins (-30.6528 kJ/mol). These effective properties may be due to the presence of carbonyl and alcoholic-OH functional groups.

Key Words: *Molecular docking studies, Cucumis trigonus Roxb, Hepatocarcinoma, Alpha-antitrypsin, CytochromeP^{450}, Silymarin.*

INTRODUCTION

Liver is one of the largest organs in human body. It is involved with almost all the biochemical pathways to growth, fight against disease, nutrient supply, energy provision and reproduction [1]. Nowadays, liver disease is a serious global health problem. Liver injury occurs because of its exposure to different agents, like chemicals, alcohols, viruses and auto-immune diseases [2]. Long-term heavy consumption of alcohol plays a major role in the development of alcohol-related liver damage [3]. Ethanol is metabolized into cytotoxic acetaldehyde by alcohol dehydrogenase enzyme in the liver and acetaldehyde is oxidized to acetate by aldehyde oxidase or xanthine oxidase giving rise to reactive oxygen species (ROS) via cytochrome P^{450} 2E1 (CYP 2E1) [4]. Therefore, search for newer drugs (with minimum side effects) obtained from traditional medicines continues. Among these herbs, *Cucumis trigonus* has been widely used for the treatment of jaundice and other hepatic disorders [5, 6].

Molecular Docking has become a valuable technique for structure-based drug discovery that predicts the preferred orientation of one molecule to a second when bound to one another to form a stable complex which in turn predicts the strength of association or binding affinity between these molecules [7]. Most of the discoveries in the past were by identifying the active ingredient present in traditional remedies [8]. Prediction of interaction energies between ligand and receptor has been a major challenge for drug docking. Now a days applying computational methods for drug discovery and development are increasingly gaining in popularity, implementation and appreciation [9].

In the present investigation molecular docking studies of the phytochemical constituents isolated and identified from the ethanolic extract of the fruits of *Cucumis trigonus* Roxb. have been carried out using the novel target receptor of hepatocarcinoma, Alpha-antitrypsin and a commonly used hepatoprotective receptor CytochromeP^{450} by using the Flex X.

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MATERIAL AND METHODS

Collection of plant materials:

The fruits of *Cucumis trigonus* was 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 (Reg.No. of the Authentification Certificate: 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 isolated and identified from the ethanolic extract of the fruits of *Cucumis trigonus* Roxb. were ascertained [10].

Nine phytochemical constituents *viz.* demeclocycline, glycodeoxycholic acid, 3α,7α,12α-trihydroxycoprostanic acid, chlortetracycline, azafrin methyl ester, giganteumgenin N, phorbol 12,13-dihexanoate, astaxanthin, tetrahydrospirilloxanthin have been isolated and identified from the ethanolic extract of the fruits of *Cucumis trigonus.* by GC-MS [11] analysis.

Potential targets and binding site:

The 3D structures of hepatic cancer potential drug targets such as Alpha antitrypsin (7API), Cytochrome P450 (2J0D), receptors were retrieved from PDB database [12]. 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 was predicted by using Flex X docking program [13].

Ligand generation:

The 2D structures of phytochemical constituents from the ethanolic extract of the fruits of *Cucumis trigonus* were drawn in ACD-Chemsketch [14] 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 [15].

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^{03} 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 mentioned receptors as docked complexes were analyzed by the pose-view of Lead IT [16].

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. Thus in the present study a novel receptor, Alpha-antitrypsin and a commonly employed receptor, CytochromeP450 were selected as a potential drug targets of HCC. The 3D structures of Alpha antitrypsin and Cytochrome P450 were determined and the molecular docking studies of ethanolic extract of the fruits of *Cucumis trigonus* have been performed.

The receptors Alpha antitrypsin and Cytochrome P450 were considered as the potential drug targets of HCC and their 3D structures were retrieved from Protein Databank (**Fig. 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.

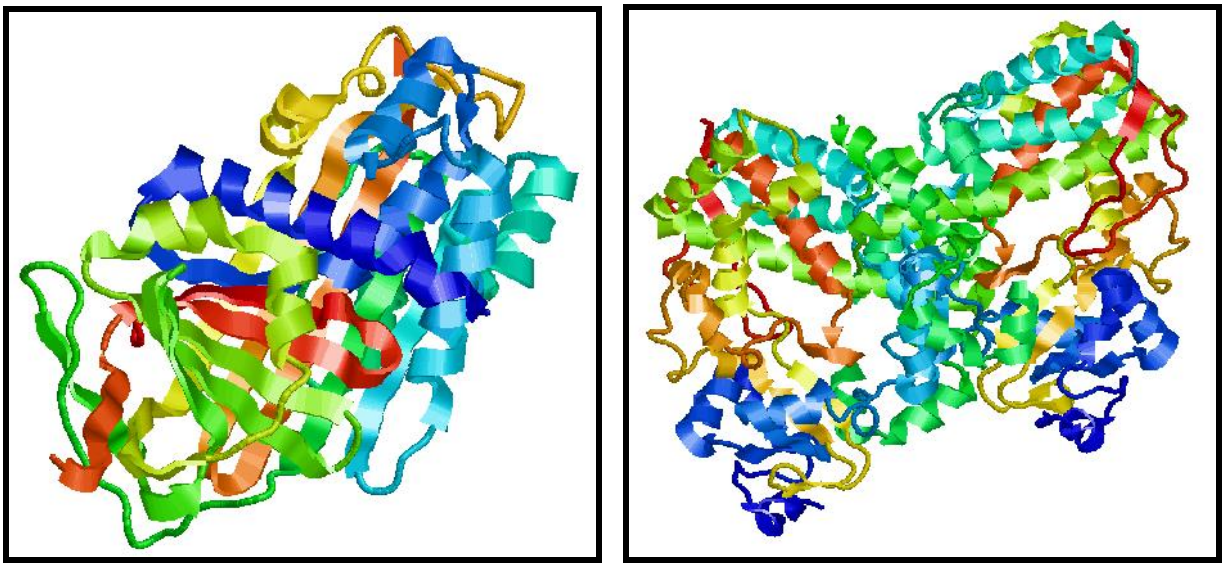


Fig. 1: 3D Structures of A) Alpha antitrypsin, B) Cytochrome P450

The 2D structures of the phytochemical constituents from the ethanolic extract of the fruits of *Cucumis trigonus* Roxb. is presented in **Table. 1**. The docking interactions between the binding site amino acids of Alpha antitrypsin and Cytochrome P450 and the 10 ligand molecules are presented in **Table. 2**. Among the nine phytochemical constituents isolated and identified from the ethanolic extract of the fruits of *Cucumis trigonus,* Silymarin (Std) is found to be the best docked ligand followed by Glycodeoxycholic acid with Alpha antitrypsin (**Fig. 2**) and Glycodeoxycholic acid is found to be the best docked ligand followed by Silymarin (Std) with Cytochrome P450 (**Fig. 3**). The results of hydrogen bonding and Hydrophobic interactions of ligand molecules with Alpha antitrypsin and Cytochrome P450 are presented in **Table. 3**.

Table No. 1: 2D Structures of phytochemical constituents isolated and identified from the ethanolic extract of the fruits of *Cucumis trigonus* Roxb.

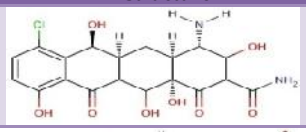
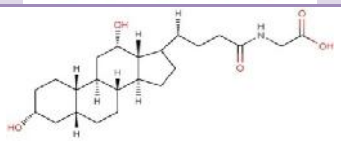
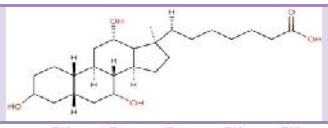
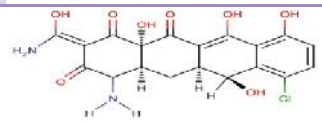
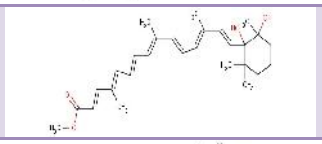
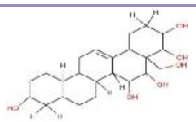
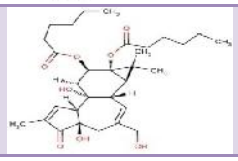
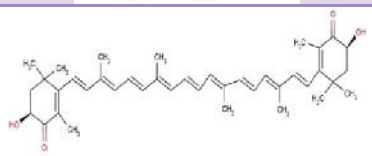
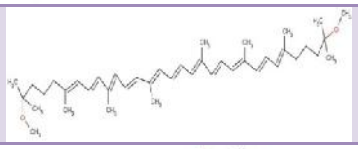
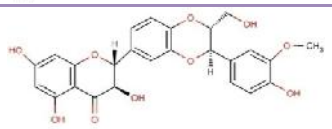
S. No.	Ligands	2D structure
1	Demeclocycline	
2	Glycodeoxycholic acid	
3	3α,7α,12α -trihydroxycoprostanic acid	
4	Chlortetracycline	
5	Azafrin methyl ester	
6	Giganteumgenin N	
7	Phorbol 12,13-dihexanoate	
8	Astaxanthin	
9	Tetrahydrospirilloxanthin	
10	Silymarin (Std)	

Table No. 2: Docking score of phytochemical constituents isolated and identified from the ethanolic extract of the fruits of *Cucumis trigonus* Roxb. with Alpha-antitrypsin and Cytochrome P^{450}

S. No	Ligands	Alpha -antitrypsin	Cytochrome P^{450}
1	Demeclocycline	-	-11.9172
2	**Glycodeoxycholic acid**	**-16.1131**	**-30.6528**
3	3α,7α,12α -trihydroxycoprostanic acid	-	-
4	Chlortetracycline	-	-12.1871
5	Azafrin methyl ester	-1.5687	-8.6125
6	Giganteumgenin N	-0.1752	-1.6609
7	Phorbol 12,13-dihexanoate	-9.6369	-5.3408
8	Astaxanthin	-	-4.7586
9	Tetrahydrospirilloxanthin	-	-4.5825
10	**Silymarin (Std)**	**-21.2777**	**-20.6443**

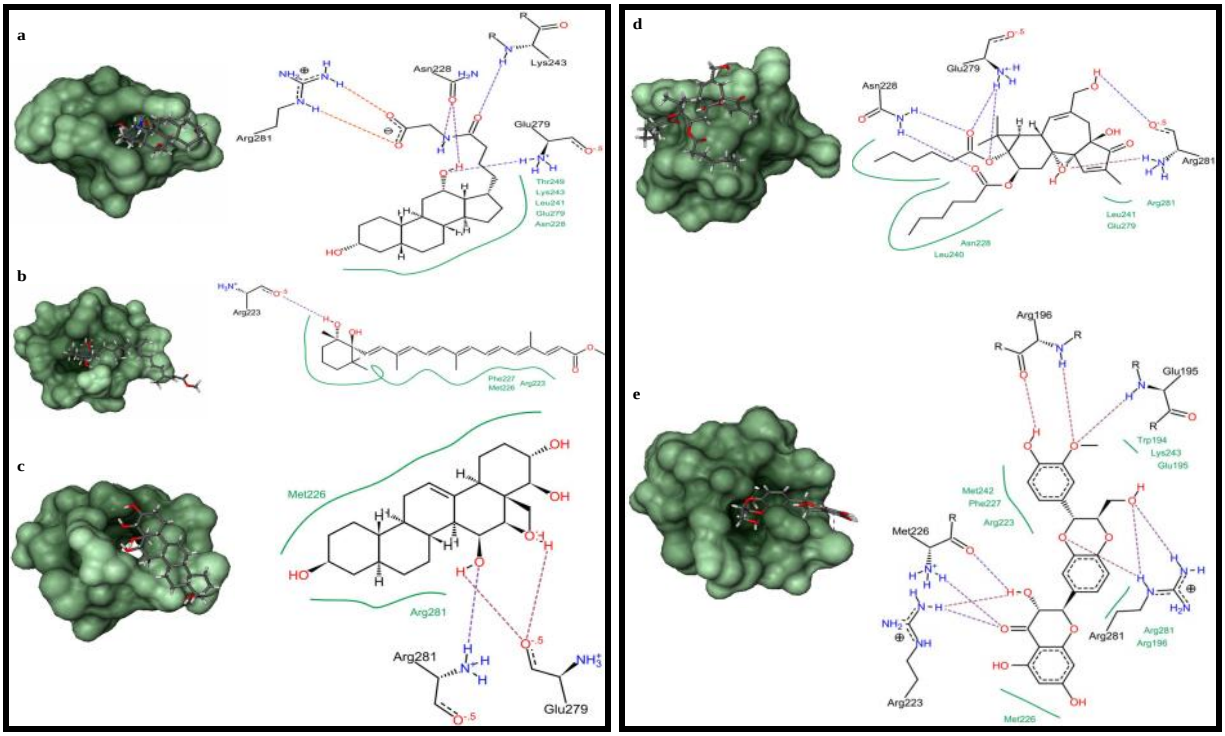


Fig. 2: Hydrogen bonding and hydrophobic interactions of phytochemical constituents isolated and identified from the ethanolic extract of the fruits of *Cucumis trigonus* Roxb. with Alpha antitrypsin
a) Glycodeoxycholic acid b) Azafrin methyl ester c) Giganteumgenin N d) Phorbol 12,13-dihexanoate e) Silymarin (Std).

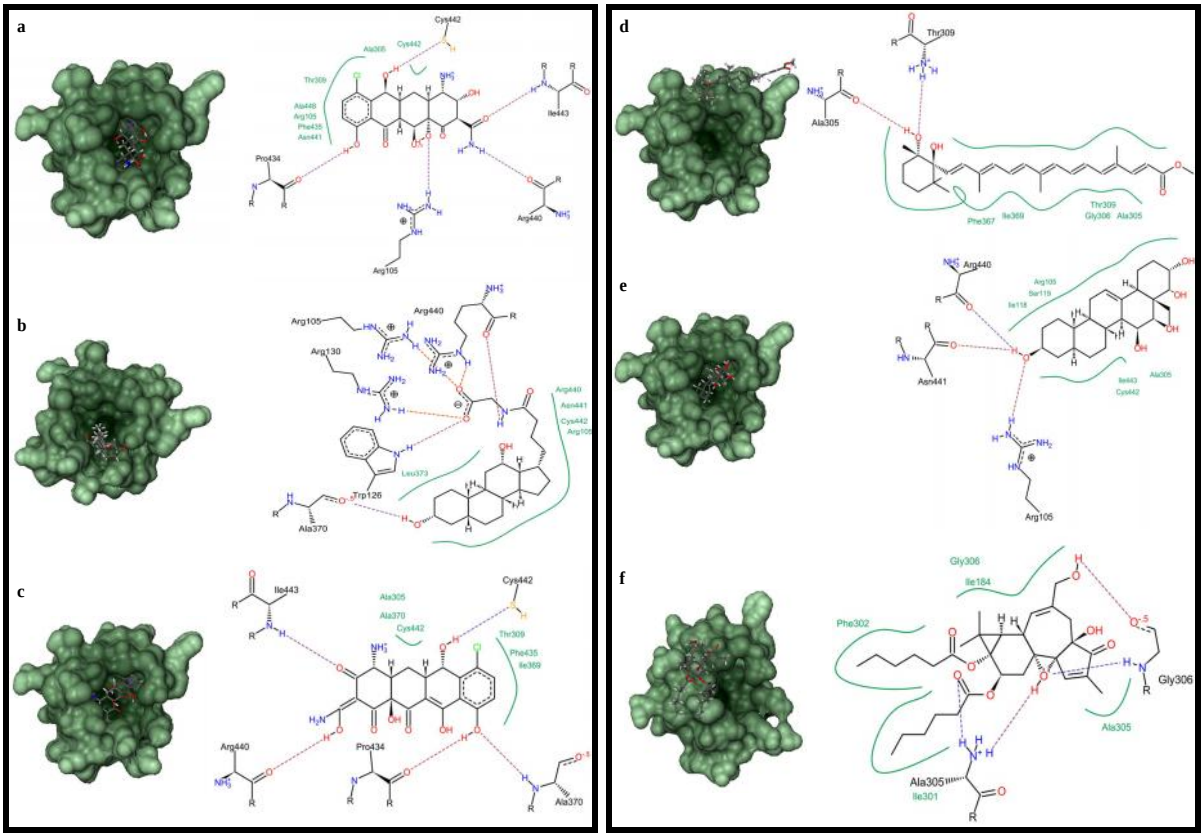


Fig. 3: Hydrogen bonding and hydrophobic interactions of phytochemical constituents isolated and identified from the ethanolic extract of the fruits of *Cucumis trigonus* Roxb. with Cytochrome P^{450}.
a) Demeclocycline b) Glycodeoxycholic acid c) Chlortetracycline d) Azafrin methyl ester e) Giganteumgenin N f) Phorbol 12,13-dihexanoate g) Astaxanthin h) Tetrahydrospirilloxanthin i) Silymarin (Std)

Table No. 3: Hydrogen bonding and hydrophobic interactions of phytochemical constituents isolated and identified from the ethanolic extract of the fruits of *Cucumis trigonus* Roxb. with Alpha-antitrypsin and Cytochrome P^{450}.

S. No.	Ligands	Alpha -antitrypsin		Cytochrome P^{450}	
		Hydrogen bonding interaction	Non bonded interaction	Hydrogen bonding interaction	Non bonded interaction
1	Demeclocycline	-	-	Pro 434, Cys 442, Arg 105, Ile 443, Arg 440	Asn 441, Phe 435, Arg 105, Ala 448, Thr 309, Ala 305, Cys 442
2	Glycodeoxycholic acid	Arg 281, Asn 228, Lys 243, Glu 279	Thr 249, Lys 243, Leu 241, Glu 279, Asn 228	Arg 105, Arg 440, Arg 130, Ala 370, Trp 126	Leu 373, Arg 440, Asn 441, Cys 442, Arg 105
3	3α,7α,12α –trihydro xycoprostanic acid	-	-	-	-
4	Chlortetracycline	-	-	Arg 440, Ile 443, Pro 434, Cys 442, Ala 370	Ala 305, Ala 370, Cys 442, Thr 309, Phe 435, Ile 369
5	Azafrin methyl ester	Arg 223	Phe 227, Met 226, Arg 223	Ala 305, Thr 309	Phe 367, Ile 369, Thr 309, Gly 306, Ala 305
6	Giganteumgenin N	Arg 281, Glu 279	Met 226, Arg 281	Arg 440, Asn 441, Arg 105	Arg 105, Ser 119, Ile 118, Ile 443, Cys 442, Ala 305
7	Phorbol 12,13-dihexanoate	Asn 228, Glu 279, Arg 281	Leu 240, Asn 228, Leu 241, Glu 279, Arg 281	Ala 305, Gly 306	Ile 301, Phe 302, Gly 306, Ile 184, Ala 305
8	Astaxanthin	-	-	-	Phe 137, Met 452, Phe 271, Phe 447
9	Tetrahydrospirilloxanthin	-	-	-	Phe 271, Phe 302, Phe 137, Ile 301, Ser 119, Ile 118
10	Silymarin (std)	Arg 223, Met 226, Arg 281, Glu 195, Arg 196	Met 242, Phe 227, Arg 223, Met 226, Arg 281, Arg 196, Trp 194, Lys 243, Glu 195	Asn 441, Arg 105, Ile 443, Arg 375	Cys 442, Gly 444, Ala 370, Phe 302, Ile 118, Ile 443, Ala 305, Arg 105, Ser 119, Asn 441, Leu 373

The protein, Alpha antitrypsin is a 52 kDa molecule produced primarily in hepatocytes and released into the blood circulation by the liver [17]. The highest docking interactions score (-21.2777 kJ/mol) was observed for Silymarin (Std) with the alpha antitrypsin receptor. The compounds, Demeclocycline, 3α, 7α, 12α-trihydroxycoprostanic acid, Chlortetracycline, Astaxanthin, and Tetrahydrospirilloxanthin do not exhibit any binding within the alpha antitrypsin active site. The best docking interactions of Silymarin (Std) is favored by the formation of hydrogen bond with Arg 223, Met 226, Arg 281, Glu 195, Arg 196. The hydrophobic interactions are contributed by Met 242, Phe 227, Arg 223, Met 226, Arg 281, Arg 196, Trp 194, Lys 243, Glu 195. The binding of remaining phytochemical constituents which exhibited the docking score ranging from -21.2777 kJ/mol to -0.1752 kJ/mol followed by Glycodeoxycholic acid which exhibited the dock score of -16.1131 kJ/mol. The interactions is favored by Arg 281, Asn 228, Lys 243, Glu 279 by hydrogen bond formation and hydrophobic formations by the means of Thr 249, Lys 243, Leu 241, Glu 279, Asn 228. It is observed that the NH group of the amino acid and the C=O present in the phytochemical constituents favors 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 the alpha antitrypsin.

The Cytochrome P^{450} is a group of heme-containing enzymes embedded primarily in the lipid bilayer of the endoplasmic reticulum of hepatocytes. It takes part in the metabolism of many drugs, steroids and carcinogens [18]. The highest docking interactions score (-30.6528 kJ/mol) was observed for Glycodeoxycholic acid with the Cytochrome P^{450} receptor. The compound, 3α, 7α, 12α-trihydroxycoprostanic acid do not exhibit any binding within the cytochtomeP^{450} active site. The best docking interactions of Glycodeoxycholic acid is favored by the formation of hydrogen bond with Arg 105, Arg 440, Arg 130, Trp 126 and Ala 370. The hydrophobic interactions are contributed by Leu 373, Arg 440, Asn 441, Cys 442 and Arg 105. The binding of remaining phytochemical constituents which exhibited the docking score ranging from -30.6528 kJ/mol to -1.6609 kJ/mol. The well known hepatic cancer drug, Silymarin which exhibited the dock score of -20.6443 kJ/mol. The interactions is favored by Asn 441, Arg 105, Ile 443 and Arg 375 by hydrogen bond formation and hydrophobic formations by the means of Cys 442, Gly 444, Ala 370, Phe 302, Ile 118, Ile 443, Ala 305, Arg 105, Ser 119, Asn 441, Leu 373. Interestingly it is observed that the amino acid, Arginine (Arg 105) is found to be conserved in all the cases of docking interactions of the phytochemical constituents. The docking study implies that the conserved amino acid, Arginine (Arg 105) in the active site of Cytochrome P^{450} receptor plays a crucial role by hydrogen bond interactions and the amino acids, Leu 373, Asn 441, Cys 442 and Arg 105 for the non bonded interactions. It is observed that the NH group of the amino acid and the C=O 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, Arginine (R), Leucine (L), Aspargine (N) and Cysteine (C) are to be considered for enhancing the hepatoprotective activity of the phytochemical constituents against the Cytochrome P^{450}.

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 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, alpha antitrypsin and cytochrome P^{450}. The results indicate that the molecular modeling is a valuable tool for predicting biological activity of phytochemical constituents. The analysis of the docking result allowed us to know the efficiency of the natural bioactive compounds to control the liver toxicity.

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How to cite this article:

Subarayan Bothi Gopalakrishnan et al.,: Evaluation of Phytochemical Constituents of *Cucumis trigonus* Roxb. for their Hepatoprotective Activity by Molecular Docking studies, J. Pharm. Res., 2015; 4(5): 182-187.

Conflict of interest: The authors have declared that no conflict of interest exists.

Source of support: Nil