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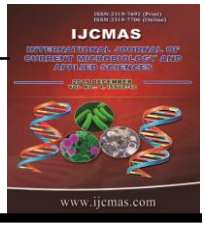

Original Research Article

A Study on *in-silico* Analysis of Phytochemicals targeting the proteins of Hepatitis B and C Virus

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Keywords

in-silico Analysis, Phytochemicals, Hepatitis B and C Virus

A B S T R A C T

Compounds from three medicinal plants effective against hepatitis B and C were selected based on literature review for docking studies. The target receptor proteins of hepatitis B and C were retrieved from PDB and the ligand compounds were retrieved from pubchem NCBI. iGEM dock software tool is used for docking analysis to screen potent inhibitors of target proteins. Out of 89 compounds, 65 compounds were chosen based on ADMET properties, Lipinski's rule of five and docked for drug efficacy. The compounds from *G.glabra* showed good docking scores for both Hepatitis B and C. This study will be useful in future for designing novel drugs for the treatment of liver diseases.

Introduction

Hepatitis B & C viruses are the most common cause of chronic disorders which leads to cirrhosis and hepato cellular carcinoma (Crockett et al., 2005). It is persisting to be one of the major global health problems due to the emergence of drug resistance **(**Chan Ran You et al in 2014**).** This urges the need for an alternative treatment to infections.

Several medicinal plants have been used for treating potential liver problems (Thyagarajan et al., 2002). *Glycyrrhiza glabra* is one such plant used as complementary & modern medicine as reported by Saxena in 2005. Glycyrrhizin from *Glycyrrhiza glabra* plays an important role in treating HCV infection (Ashfaq *et al.*, 2011). Phytochemicals of *Terminalia chebula* possess potent antiviral activity (Kim *et al.*, 2001).

The Ethanolic extract of *Plectranthus amboinicus* has shown hepatoprotective effect (Shenoy et al., 2012). The aim of drug designing is made simple with bioinformatic tools. Hence, the present work relies on the in-silico information of the Medicinal plants against Hepatitis B & C Virus.

Materials and Methods

Target proteins

Crystal structures of target proteins of Hepatitis B virus such as HBx protein, (PDB ID: 3 MSH) $GIPC_2$, (PDB ID: 3GGE) Mahira Arooj et al., in 2012 and (PDB ID: 2DXS), (PDB ID: 4E06) NS5B RNA polymerase protein from hepatitis C Virus was obtained from RCSB Protein data bank.

Natural Compounds from medicinal plants

The Structure of Medicinal plant compounds from *Glycyrrhiza glabra*, *Terminalia chebula* and *Plectranthus amboinicus* were chosen for study based on review of literature (Table 1).

The compounds were retrieved from pubchem databases. These compounds were saved in (.sdf) file format & converted into (.mol) format by using MedChem Designer.

Out of 89 compounds, 65 compounds satisfied the ADMET profile which analysed the adverse properties of Absorption, distribution, metabolism, toxicity of the compounds.

Table.1 The Selected Natural compounds form medicinal plants based on ADMET properties

S.No	Plant Name	Compound	Reference
1.	***Glycyrrhiza glabra***	**Daidzin (daidzein-7-glucoside)**	khalaf et al., 2010.
2.		**Ononin (formononetin -7-glucoside**	
3.		Formononetin(7-hydroxy-4'-Methoxyisoflavone)	
4.		Genistein(4',5,7-trihydroxyisoflavone)	
5.		Coumestrol	
6.		5-(Hydroxymethyl)-2-Furan carboxaldehyde	
7.		2,4,5-Trimethyl-1,3-Dioxolane	
8.		N,1-Dimethyl-4-piperidinamine	
9.		3-Isopropyl alanine	
10.		2-Methoxy-4-vinylphenol	
11.		Cis2,6 Dimethyl morpholine	
12.		6-Methoxy-8-methyl-8-azabicyclo(3.2.1)octan-3-ol	
13.		1-Dodecanol	
14.		7-Methoxy-2-benzofuranylmethyl ketone	
15.		2-pyridine carboxylic acid	
16.		Benzoic acid	
17.		Salicylic acid	

18.		**Hexadecanoic acid ,1-methyl ethyl ester**	
19.		N-methyl-4-(4-methyl-1-phthalazinylamino)-Benzamide	
20.		Dodecanoic Acid	
21.		Furan-2-carboxaldehyde(Furfural)	
22.		Tetrahydrofufuryl alcohol	Vijayalakshmi et al., 2013
23.		Glabridin	
24.		Liquiritigenin	
25.		Licoisoflavone	
26.		Hymecromone	
27.		Herniarin	
28.		Spathulenols	
29.		**Colecoxib**	kaur at al., 2009.
30.		**Rofecoxib**	
31.		S)-(+)-2-Amino-3-methyl-1-butanol	
32.		Ethyl pipecolinate	
33.		Cyclopropanecarboxylic acid,1-amino-	
34.		2,5-Pyrrolidinedione	
35.		Flamenol	
36.		Conhydrin	
37.		n-Decanoic acid	
38.		Z-2-Dodecenol	
39.		2,4,6-Trimethyl-1-nonene	
40.		**1,2-Benenedicarboxylic acid,bis(2-methylpropyl)ester**	Nandagopal et al., 2014
41.	***Terminalia chebula***	Decanoic acid,ethyl ester	
42.		2-Propenoicacid, 2-(dimethylamino)ethyl ester	
43.		1,Cyclohexylnonene	
44.		Noxiptiline	
45.		1,14-Tetradecanediol	
46.		Glycine,N-(2-methyl-1-oxo-2-butenyl)-,methyl ester,(E)-	
47.		4-Methoxycarbonyl-4-butanolide	
48.		Benzeneacetaldehyde	
49.		Butane,1,1-diethoxy-	
50.		Glycerin	
51.		Phenol	Elamparithi et al., 2011
52.		1-Piperidineacetonitrile	
53.		Levoglucosenone	
54.		Piperazine,1-(aminoacetyl)-	

55.		Resorcinol	
56.		Cycloheptanone	
57.	***Plectranthus amboinicus***	Linalool	Asiimwe et al., 2014
58.		Nerol acetate	
59.		Geranyl acetate	
60.		Carvacrol	
61.		ɤ-Terpinene	
62.		Nerol	
63.		β- Cymene	
64.		β -Myrcene	
65.		β -Ocimene	

iGEM Docking

The compounds were screened by iGEM docking software to study the inhibitors of target proteins. This software runs for 70 generations to know the best score of the compound. Based on Binding energy, Hydrogen bonding (H), and electrostatic (E) and Vander Waal's interactions, post screening analysis was carried out. (Balavignesh et al., 2013) By the combination of binding energy and pharmacological interactions, suitable compounds are selected.

Results and Discussion

65 compounds were screened for insilico analysis using iGEM Docking software. The best compound interacting with receptor proteins of both Hepatitis B and Hepatitis C are summarized in Table 2.

From the protein-ligand interactions, the phytocompound Daidzin, is found to have best fitness scores and also exhibit drug likeliness followed by Ononin, Colecoxib and Rofecoxib from *Glycyrrhiza glabra* and1, 2-benzene di carboxylic acid, bis(2-methyl propyl ester) from *Terminalia chebula* againt HBx proteins of hepatitis B(PDB ID: 3 MSH) .Fei Su et al., in 1996 reported that the HBx protein is a small polypeptide essential for viral infection and play a role in development of HCC during chronic Hepatitis B virus infection..Another target protein selected for the study is GIPC2 (PDB ID: 3GGE) - a protein that interacts with HBV core and participate in intravesicular transport (Razanskas et al., 2003). The lead compounds active against this protein are Colecoxib, Daidzin, Rofecoxib, Ononin and Licoisoflavone from *Glycyrrhiza glabra* showing good scores against Hepatitis B.

The most active ligand docks into the binding pockets of enzyme NS5B polymerase are Ononin, followed by Rofecoxib Diadzin, Colecoxib and Glabridin from *Glycyrrhiza glabra*. Whereas Hexa decanoic acid,1-methyl ester, Daidzin, Colecoxib, Ononin and Licoisoflavone are the compounds from

Glycyrrhiza glabra showed best docked poses to be effective in inhibiting another target protein of HCV NS5B Polymerase enzyme. Balavignesh et al., 2013 in his study reported that it is a RNA dependent RNA polymerase enzyme which is essential for viral replication of hepatits C virus. In the present study, the ligands from *Glycyrrhiza glabra* namely Daidzin, Ononin, Colecoxib and Rofecoxib are the lead compounds of the inhibitors of both Hepatitis B and Hepatitis C respectively

Table.2 Top five compounds showing good scores against Hepatitis B and Hepatitis C virus

S.no	Plant name	Compound	ID number	HBV		HCV	
				PBD ID: 3MSH	PBD ID: 3GGE	PBD ID: 2DXS	PBD ID: 4EO6
1	*G.glabra*	Daidzin	107971	-105.7	**-119.81**	-111.3	-107.4
2	*G.glabra*	Ononin	442813	-101.6	-109.46	**-139.2**	-104.4
3	*G.glabra*	Colecoxib	2662	-99.3	**-130.04**	-107.6	-106.3
4	*G.glabra*	Rofecoxib	5090	-91.1	-110.12	**-115.9**	-
5	*G.glabra*	Licoisoflavone	5481234	-	**-107.15**	-	102.4
6	*G.glabra*	Hexadecanoic acid,1-methyl ester	985	-	-	-	-109.8
7	*T.chebula*	1,2-benzene di carboxylic acid,bis (2-methyl propyl ester)	6782	-88.8	-	-	-

The structures of post screening analysis for the top two compounds of the proteins of hepatitis B and C virus are as follows.

Fig.1 Interactions between (PDB ID: 4EO6) & Hexa decanoic acid, 1-methyl ester (985)

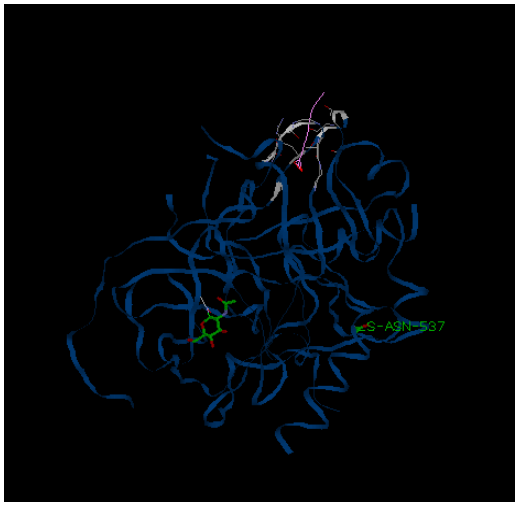


Fig.2 Interactions between (PDB ID: 4EO6) & Daidzin (107971)

Fig.3 Interactions between (PDB ID: 2DXS) & Ononin (442813)

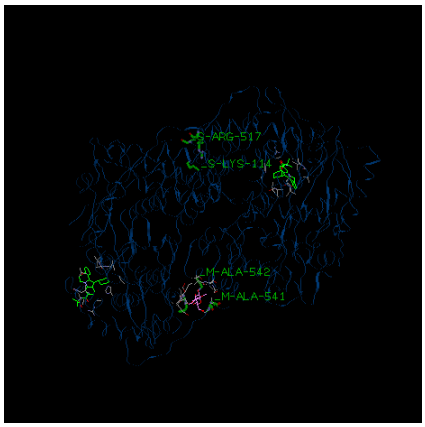


Fig.4 Interactions between (PDB ID: 2DXS) & Rofecoxib (5090)

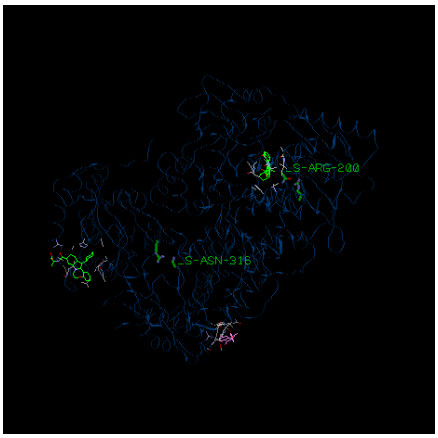


Fig.5 Interactions between (PDB ID: 3GGE) & Colecoxib (2662)

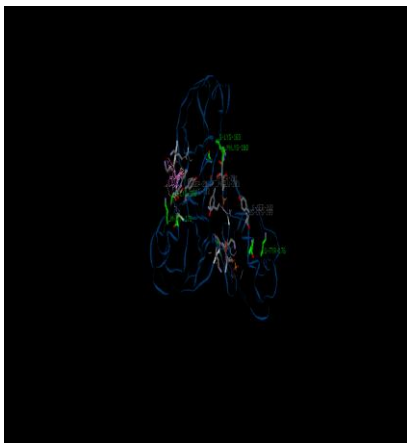


Fig.6 Interactions between (PDB ID: 3GGE) & Daidzin (107971)

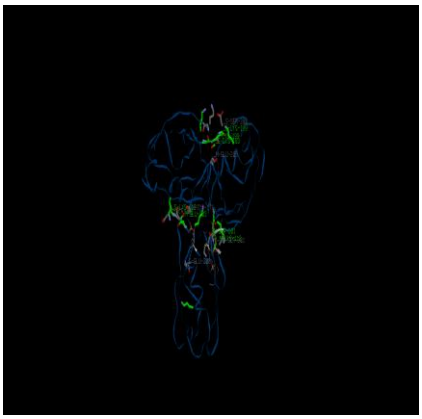


Fig.7 Interactions between (PDB ID: 3MSH) & Daidzin (107971)

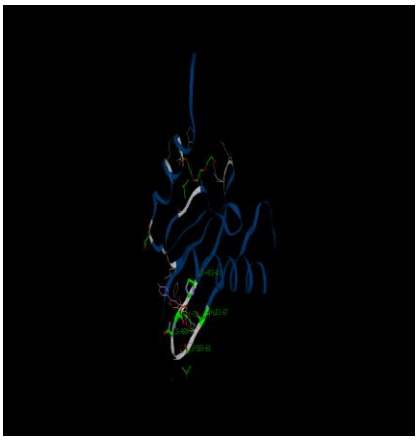
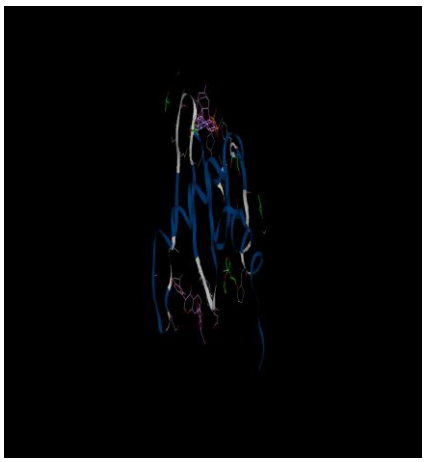


Fig.8 Interactions between (PDB ID: 3MSH) & Ononin (442813)



In conclusion, Insilco analysis is the safest method of screening drugs particularly in viral infections without any side effects. It is also a cheap & reliable method in preliminary identification of the active compounds. Omprakash et al., 2011.From the results of iGEM Docking analysis, it was found that of all the compounds tested from different medicinal plants, the compounds from *G. glabra* were found to be effective against both Hepatitis B & C followed by the compound of *Terminalia chebula*. The compounds from *Plectranthus amboinicus* did not possess best docked scores in both Hepatitis B and Hepatitis C. In the present study, it was investigated that the compounds from Glycyrrhiza glabra and Terminalia chebula showing good scores were proved to be active compounds which possess anti hepatitic activity for both Hepatitis B & C.

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