ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Virtual Screening of natural HBV capsid assembly inhibitor using Molegro Virtual Docker for identification of potential lead compound for Hepatitis B.

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ABSTRACT

HBV core protein is a small protein that self-assembles to form the viral capsid. It modulates almost every step of the viral life cycle in infected cells. HBV core protein is an excellent target for the development of new, virus-selective, safe and effective antiviral agents for treatment options hepatitis B disease. The study aims to find HBV capsid assembly inhibitor from bioactive compounds derived plants through analysis of binding free energy with HBV core protein using molecular docking simulation. The 3D structure of the core protein of HBV was downloaded from the PDB (Code ID:5E0I). The structure of 500 ligands was imported from herbal database at herbaldb.farmasi.ui.ac.id (Compounds: HERBALD00000001-HERBALD00004745). The docking simulation was done using Molegro Virtual Docker ver 6.0 program. Validation of the docking method was performed by redocking the reference ligand NVR10-001E2 that has been co-crystal in the HBV core protein 5E0I. The docking of bioactive compounds was performed in cavity 2 Coordinate x =146.31, y=6.43, z=123.97, dimension 15A. From 28 compounds with the reranking score less than -120 kcal/mol, there were five compounds indicated a free violation of Lipinski's rules of five. The compounds were Dihydroanhydropodorhizol, Curcumin, Mulberrin, Nobiletin and 5-Hydroxy-7,8-dimethoxyflavone 5-glucoside. Curcumin was a compound that is potentially used as a lead compound because of the availability, convenience, and relatively low price. Curcumin has potential lead compound as an HBV capsid assembly inhibitor for development hepatitis B drug candidates.

Keywords: Hepatitis B virus, HBV core protein, natural compounds, herbal database, Molegro Virtual Docker.

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ISSN: 0975-8585

INTRODUCTION

The hepatitis B virus (HBV) infection is a worldwide health problem and infects more than 350 million individuals worldwide. HBV causes acute and chronic hepatitis. In many cases HBV can lead to lifelong illness, liver cirrhosis, liver cancer, hepatocellular carcinoma, liver failure and death [1], [2], [3]. Chronic HBV infection is the dominant global cause of hepatocellular carcinoma. There are 55% of cases worldwide and 80% or more in the eastern Pacific region and sub-Saharan Africa. [1], [4]. The risk of developing chronic HBV infection decreases with age at infection. The data showed 90% of people with hepatitis B were infected when perinatally up to 6 months, 20%–60% between the ages of 6 months and five years and 25% of people who acquire HBV as children will develop primary liver cancer or cirrhosis as adults. Recent Global Burden of Disease estimates indicates a high morbidity and mortality attributable to chronic HBV, despite decreases over the past decades [5].

HBV is a small enveloped DNA virus whose genome has only four open reading frames. The simplicity of the virion correlates with a complexity of functions of viral proteins. HBV core protein is a small protein that self-assembles to form the viral capsid. In an infected cell, it modulates almost every step of the viral lifecycle. It binds to nuclear viral DNA and affects its epigenetics. HBV core protein correlates with RNA specificity, assembles specifically on a reverse transcriptase-viral RNA complex. HBV core protein participates in the regulation, signals the completion of reverse transcription to support virus secretion. It carries both nuclear localization signals and HBV surface antigen (HBsAg) binding sites.

The HBV core protein is a viral protein with no known related protein present in human cells. It is an excellent target for the development of new, virus-selective, safe and effective antiviral agents to improve treatment options for this disease. It consists of 183-185 amino acids that form an N-terminal (amino acids 1-149) capsid assembly domain and a C-terminal nucleic acid binding domain (amino acids 150-185). The viral capsid in infectious HBV particles is formed from 120 copies of assembled core protein dimers enclosing the viral DNA [6]. To date, interferon and nucleotide analogues are the approved treatments for chronic HBV infection, but the side effects and drug resistance limit the use of these available options (Zhao, 2010). The further development of potential antiviral therapy is still required. Small molecules that assembly with an HBV core protein domain can disrupt functional HBV capsid assembly. It can be potent inhibitors of HBV replication [7].

Plants have a key source of highly effective conventional drugs for the treatment of many forms of diseases. The actual compounds isolated from the plants may not serve as the drug, but leads to the development of potential agents. To understand the effectiveness of natural compounds as antiviral molecules, tools is needed such as a computational method that can identify and analyze active sites and suggest potential drug molecules that can bind to these sites specifically and play a vital role in the investigation of novel drug molecules.

Docking various ligands to the protein of interest followed by scoring to determine the binding affinity and to reveal the strength of interaction has become extensively used in virtual screening of large databases and lead optimization [8].

In our study, five hundred natural compounds derived plant from the herbal database were screening for HBV capsid assembly inhibitor using Molegro Virtual Docker. Various molecular structures from Herbal Data Base of the ligands were docked and scored to identify the ligands that bind similar to reference ligand binding for HBV core protein and to estimate the ligands binding affinity for its target.

MATERIAL AND METHODS

Five hundred natural compounds derived plant were imported from herbal database in www.herbaldb.fa.ui.ac.id. A single rigid crystal structure of an enzyme is used for docking studies from 25 crystal structure of HBV core protein held in the protein data Bank (PDB) accessed at the URL (http://www.rscb.org/pdb) under criteria that they had a reasonable resolution (≤ 2.8 Å), in complex with small molecule ligands. Docking studies were performed by Molegro Virtual Docker installed on a single machine running on AMD Athlon ™ II X3 445 processor with 4GB RAM with Windows 32-bit as the operating system.

Docking Simulation

Molegro Virtual Docker (MVD) Ver.6.0 was used for docking studies. MVD requires a three-dimensional structure of both protein and ligand. Validation of the docking method was performed by redocking the reference ligand NVR10-001E2 that has been co-crystal in the HBV core protein 5E0I. The docking of the reference ligand and natural compounds were performed in cavity 2 Coordinate x =146.31, y=6.43, z=123.97, dimension 15A.

Five hundred compounds have been docked into HBV core protein crystal structure, and ten independent runs were performed with the guided differential evolution algorithm, with each of these docking runs returning one solution (pose). The Moldock scoring function used by MVD is derived from the PLP scoring function originally proposed by Gehlhaar et al. and extended later by Yang et al [9].

RESULT AND DISCUSSION

To estimate the HBV capsid assembly inhibitor activity of natural compounds derived, molecular docking study was performed using Molegro Virtual Docker (MVD) Ver.6.0. We used receptor core protein of HBV (PDB code ID:5E0I) as the target protein. MVD automatically identifies potential binding sites by using its cavity detection algorithm. The cavaties within a 30 x 30 x 30 Å3 cubes centered at the experimentally known ligand position were used. The Program indentified five different binding sites of the crystal structures of the core protein of HBV complexes (Figure 1). From these five predicted cavities, the one with was selected for consideration, as it includes the bound ligand.

Validation of the docking method was performed by redocking the reference ligand NVR10-001E2 that has been co-crystal in the HBV core protein 5E0Ichain D (Cavity 2; Volume=416.768) with radius 15 Å showed an RMSD value of 1.31 and affinity energy as -132.92 kcal/mol. NVR-010-001-E2 can induce the assembly of the HBV core wild-type and Y132A mutant proteins. This compound accelerates thermostabilization of proteins by increasing Tm more than 10 ° C. It binds to dimer-interface dimer of core protein, forming new interaction surfaces that promote protein-protein interaction, induce protein assembly and increase stability. [10].

One application of molecular docking is to screen *in silico* against the protein. Five hundred compounds from herbaldb.farmasi.ui.ac.id were docked on to the protein, on the same cavity. The results concluded which confirmation produced the lowest energy state when bound to the target protein, were shown as Rerank Score. In each docking run, the best poses were selected by their MVD rerank scores, and the mean of the five rerank scores were computed as the final score for each compound [11]. The rerank score of the 28th best poses for each of docking studies of the compound from the herbal database and reference ligand to HBV core protein summarized in Table 1.

From 500 compounds, there were 28 compounds have rerank score less than -120 kcal/mol. The highest potential inhibition of HBV capsid is capsorubin through molecular docking approach with rerank score -143 kcal/mol. Capsorubin is known has molecular weight more than 500 Da, which indicated that Lipinski's rules of five has been violated.

Five compounds indicated free violation of Lipinski's rules of five. The compounds were Dihydroanhydropodorhizol, Curcumin, Mulberrin, Nobiletin and 5-Hydroxy-7,8-dimethoxyflavone 5-glucoside. Curcumin was a compound that is potentially used as a lead compound because of the availability, convenience, and relatively low price. Curcumin was known has molecular weight 368.385 Da; Log P value 2.56; 2 (two) hydrogen bond donors and 6 (six) hydrogen bond acceptors.

The interaction between reference ligand and curcumin indicated by hydrogen bonding (fig 2) and steric interactions (fig 3) between the ligand with the amino acid residues in HBV core protein. Hydrogen bond interaction of NVR10-001E2 with HBV protein core amino acid residue Trp 102, Dihydroanhydropodorhizol with residue Ser 102; Mulberin with 4 (four) amino acid residues Pro 138, Tyr 118, Thr 128 and Ser 106; Curcumin with amino acid residue Trp 102; Nobiletin with 4 amino acid residues Leu 140, Tyr 118, Trp 102 and Thr 33; 5-Hydroxy-7,8-dimethoxyflavone 5-glucoside with 4 amino acid residues Tyr 118, Leu 140, Thr 128 and Trp 102.

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Docking results also show steric interaction with score NVR10-001E2 (-109.2 kcal/mol); Nobiletin (-163.98 kcal/mol); 5-Hydroxy-7,8-dimethoxyflavone 5-glucoside (-158.44 kcal/mol); Dihydroanhydropodorhizol (-155.46 kcal/mol); curcumin (-152.92 kcal/mol) and Mulberin (-143.97 kcal/mol).

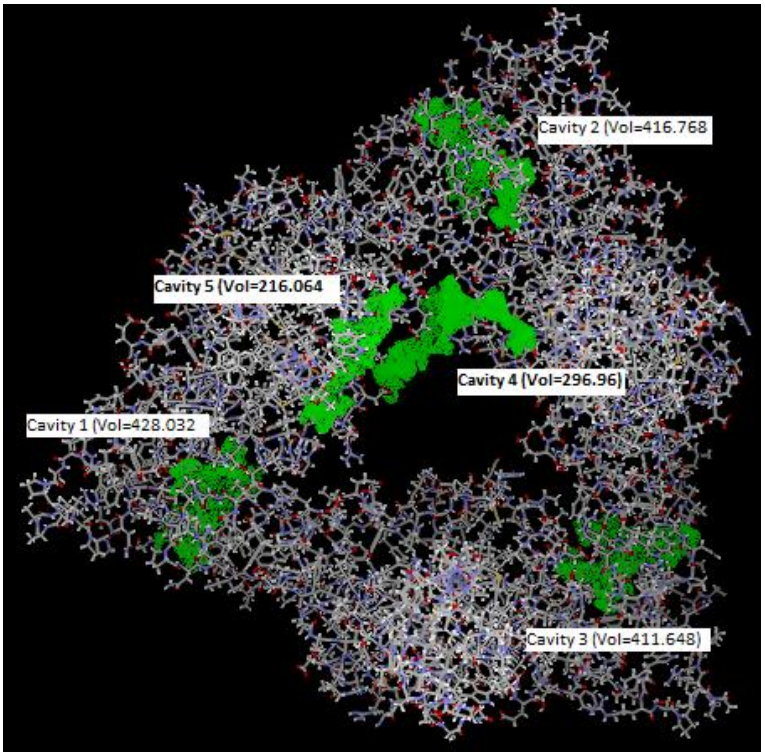
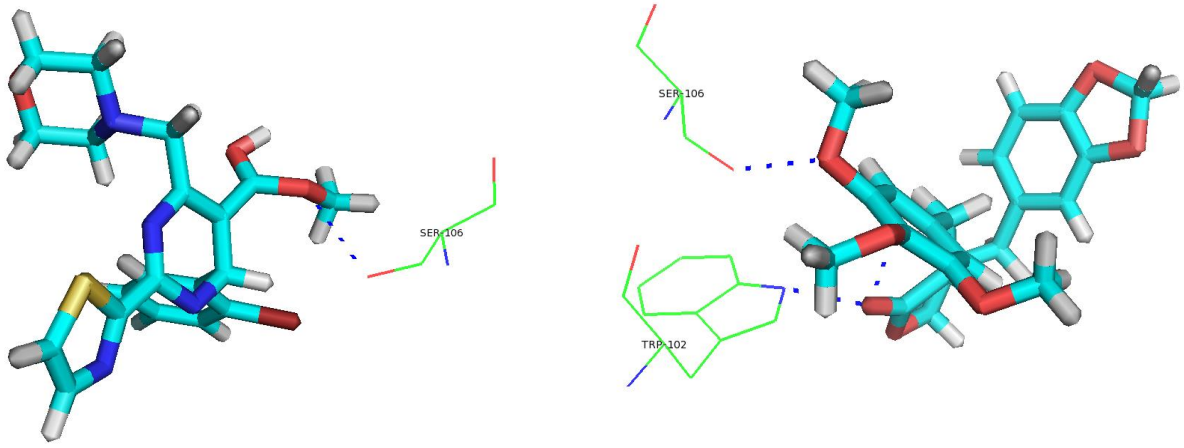


Fig 1. The five cavities in core protein HBV (PDB code 5E0I) and their calculated volumes (in Å)



(a) (b)

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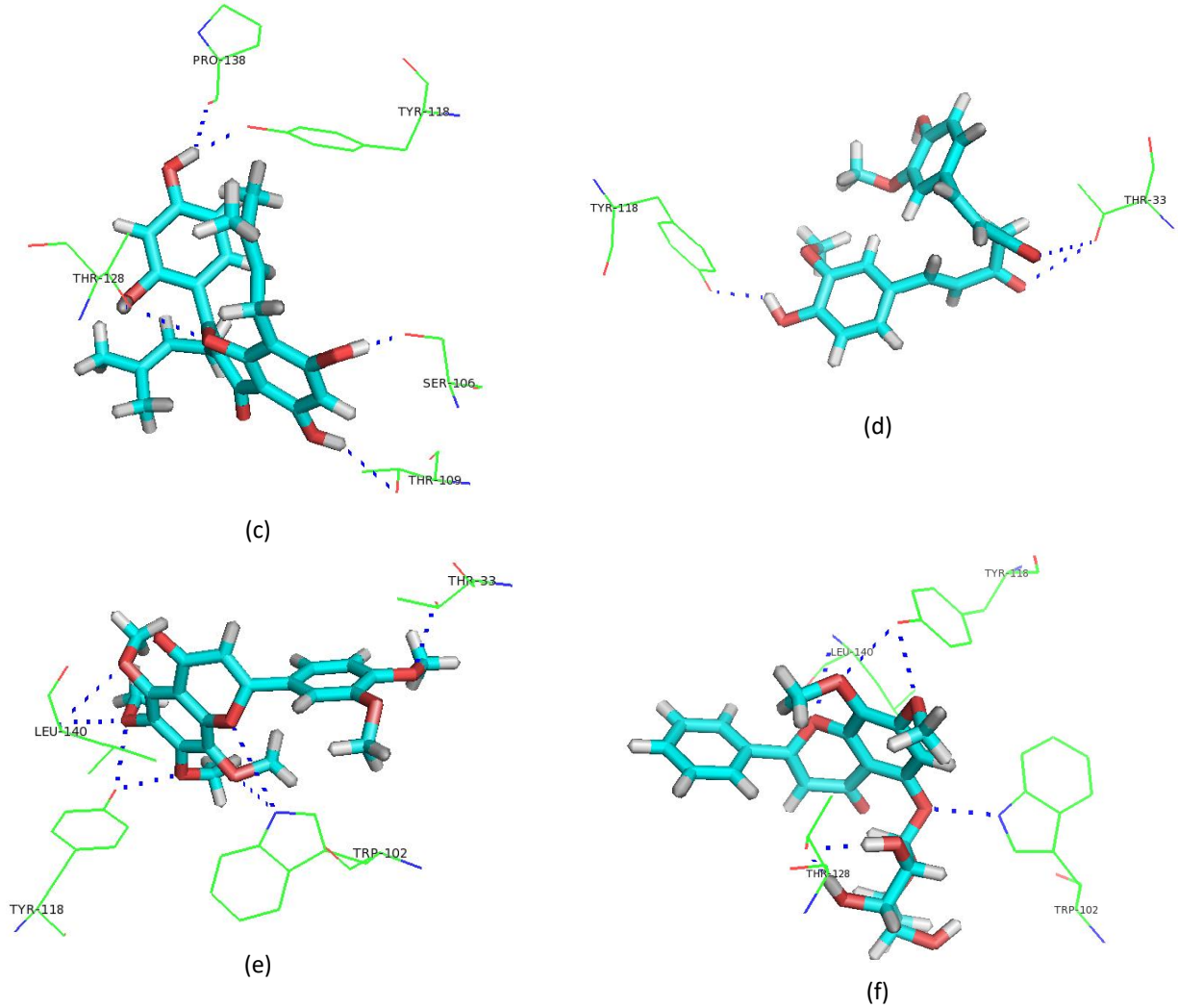
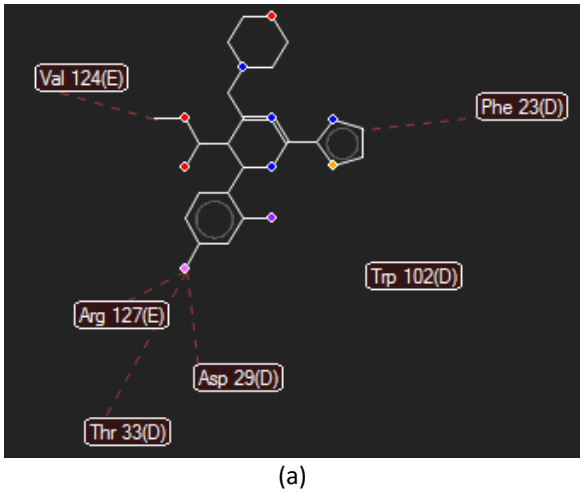
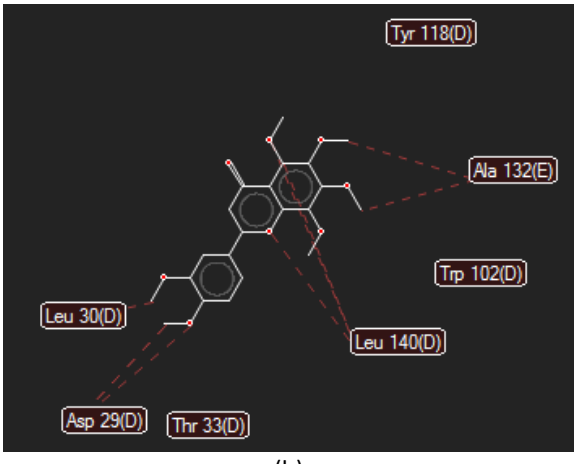


Fig 2. Hydrogen Bond Interactions of ligand NVR10-001E2 (a); Dihydroanhydropodorhizol (b); Mulberrin (c); Curcumin(d); Nobiletin (e) and 5-Hydroxy-7,8-dimethoxyflavone 5-glucoside (f) with HBV core protein (PDB ID: 5E0I).



(a)



(b)

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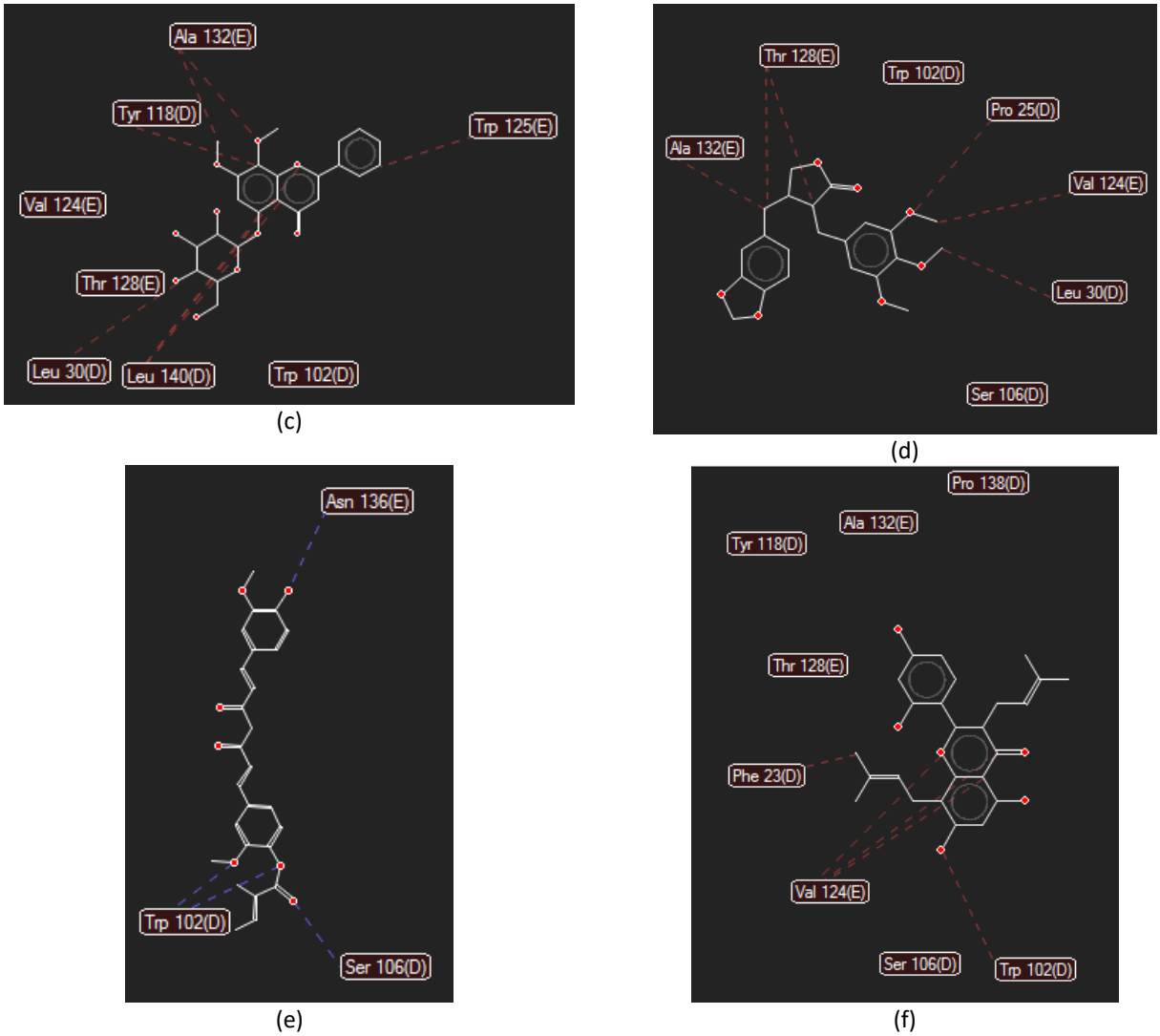

Fig 3. Steric Interactions of ligand NVR10-001E2 (a); Nobiletin (b); 5-Hydroxy-7,8-dimethoxyflavone 5-glucoside (c); Dihydroanhydropodorhizol (d); Curcumin(e);and Mulberrin (f) with HBV core protein (PDB ID: 5E0I).

Table 1. Docking Score of natural compounds from Herbal Data Base into HBV core protein (PDB ID: 5E0I)

No	Ligand	Rerank Score (kcal/mol)	HBond Score (kcal/mol)
1	Capsorubin.mol	-143.763	-7.499
2	Capsanthin.mol	-143.137	-3.476
3	Mutatochrome.mol	-141.814	0
4	Neoxanthin.mol	-140.177	-5.665
5	Violaxanthin.mol	-136.958	-1.012
6	Casuarictin.mol	-136.926	-7.993
7	Glucobrassicin.mol	-133.497	-11.112
8	Carthamone.mol	-133.227	-9.929
9	5-Hydroxy-7,8,2'-trimethoxyflavone 5-glucoside.mol	-132.946	-10.557
10	epi-Gallocatechin 3-O-gallate.mol	-132.665	-15.340
11	gamma-Carotene.mol	-130.682	0

ISSN: 0975-8585

12	Luteolin 7-(2''-p-coumaroylglucoside).mol	-130.532	-5.015
13	Antheraxanthin.mol	-130.189	-2.5
14	Asperuloside.mol	-129.127	-13.519
15	Rubixanthin.mol	-128.429	-2.5
16	Paederoside.mol	-128.118	-9.781
17	Agnuside.mol	-127.657	-15.209
18	Kuwanone H.mol	-127.374	-11.746
19	alpha-Cryptoxanthin.mol	-126.936	0
20	Cyanin.mol	-126.089	-17.897
21	Dihydroanhydropodorhizol.mol	-124.104	-2.158
22	Apiin.mol	-123.65	-8.655
23	Luteolin 3'-methyl ether 7-mannosyl-(1-2)-alloside.mol	-123.439	-10.549
24	Luteolin 7-apiosyl-(1-2)-glucoside.mol	-122.875	-17.733
25	Curcumin.mol	-121.589	-0.898
26	Mulberrin.mol	-121.179	-8.482
27	Nobiletin.mol	-120.17	-7.662
28	5-Hydroxy-7,8-dimethoxyflavone 5-glucoside.mol	-120.024	-10.836

Table 2. Physicochemical properties and RO5 Lipinski violation of of natural compounds from Herbal Data Base

No	Ligand	Molecular Weight (Da)	log P	Hydrogen Bond Donor Count	Hydrogen Bond Acceptor Count	Violation of Lipinski`s Rule
1	Capsorubin	600.88	9.11	2	4	2
2	Capsanthin	584.89	8.44	2	3	2
3	Mutatochrome	552.89	9.53	0	1	2
4	Neoxanthin	600.88	5.57	3	4	2
5	Violaxanthinl	600.88	6.04	2	4	2
6	Casuarictin	936.65	3.15	15	26	4
7	Glucobrassicin	448.46	-0.62	6	11	2
8	Carthamone	448.38	-0.71	6	11	1
9	5-Hydroxy-7,8,2'-trimethoxyflavone 5-glucoside	490.46	0.46	4	11	1
10	epi-Gallocatechin 3-O-gallate	458.37	2.07	8	3	1
11	gamma-Carotene	536.89	10.76	0	0	2
12	Luteolin 7-(2''-p-coumaroylglucoside)	594.53	0.87	7	13	3
13	Antheraxanthin	584.89	6.9	2	3	2
14	Asperuloside	414.36	-2.57	4	11	1
15	Rubixanthin	552.89	9.4	1	1	2
16	Paederoside	446.42	-1.28	4	12	1
17	Agnuside	466.44	-1.2	6	11	2
18	Kuwanone H	760.84	8.42	8	11	4
19	alpha-Cryptoxanthin	552.85	9.45	1	0	2

20	Cyanin	611.53	-2.28	11	15	3
21	Dihydroanhydropodorhizol	400.43	3.12	0	7	0
22	Apiin	564.50	-2.33	8	14	3
23	Luteolin 3'-methyl ether 7-mannosyl-(1-2)-alloside	624.55	9.4	9	16	4
24	Luteolin 7-apiosyl-(1-2)-glucoside	580.50	6.76	9	15	4
25	Curcumin	368.39	2.56	2	6	0
26	Mulberrin	422.48	4.74	4	6	0
27	Nobiletin	402.40	0.8	0	8	0
28	5-Hydroxy-7,8-dimethoxyflavone 5-glucoside	460.44	0.59	4	10	0

Five compounds indicated free violation of Lipinski's rules of five. The compounds were

CONCLUSION

The docking studies as described above provide an estimation of inhibitory activities of the docked ligand. The results showed that five compounds fit well in the active site of HBV core protein and also interact with the residues in the active site which are important for their biological activity. Therefore Curcumin has potential lead compound as an HBV capsid assembly inhibitor for development hepatitis B drug candidates.

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