

RESEARCH ARTICLE

Molecular modeling studies of repandusinic acid as potent small molecule for hepatitis B virus through molecular docking and ADME analysis

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Background: Hepatitis B virus (HBV) has affected over 300 million people worldwide which causes to induce mostly liver disease and liver cancer. It is a member of the family Hepadnaviridae which is a small DNA virus with unusual characters like retroviruses. Generally, hepatoprotective drugs provoke some side effects in human beings. For the reason, this study aims to identify alternative drug molecules from the natural source of medicinal plants with smaller quantity of side effects than those conventional drugs in treating HBV.

Methods: We developed computational methods for calculating drug and target binding resemblance using the Maestro v10.2 of Schrodinger suite. The target and ligand molecules were obtained from recognized databases. Ligand molecules of 40 phytoconstituents were retrieved from variety of plants after we executed crucial analyses such as molecular docking and absorption, distribution, metabolism, and excretion (ADME) analysis.

Results: In the docking analysis, the natural analogues repandusinic acid showed better docking scores of -14.768 with good binding contacts. The remaining bioactive molecules corilagin, furosin, nirurin, iso-quercetin and gallicocatechin also showed better docking scores.

Conclusion: This computational analysis reveals that repandusinic acid is a suitable drug candidate for HBV. Therefore, we recommend that this analogue is suitable in further exploration using *in vitro* studies.

Keywords: hepatitis B virus; phytoconstituents; molecular docking; ADMET analysis

Author summary: Hepatitis B virus affects the human beings due to the virus replication. The conventional drugs of hepatitis B virus have side effects on human and few are not responsible for that diseases complication. Hence, present computational studies found a few effective phytocompounds for hepatitis B virus which are identified based on their mode of interactions with hepatitis B viral protein molecule. Among the molecules, repandusinic acid has a better docking score and it was shown good binding affinities with the target. *In vitro* and *in vivo* evaluation will be essential and we hope this study will be helpful to proceed further with the effective drug development.

INTRODUCTION

According to world health organization (WHO), two billion people have been infected with hepatitis B virus (HBV) globally, and more than 400 million people are affected as lifetime patients [1]. Owing to severe

consequences of hepatitis the people are mostly affected by liver failure, fibrosis, cirrhosis and hepatocellular carcinoma (HCC). Whereas in India, approximately 80 million people are affected and 2,400,000 people died due to this disease complications [2].

Recently, the US Food and Drug Administration (FDA)

have approved seven drugs for the treatment of HBV. Of them, alpha-interferon and ribavirin are used together for reducing the affection of Hepatitis B virus. But, these two drugs cause several side effects like hemolytic anemia, renal failure, etc [3]. The remaining hepatitis drugs of lamivudine (3TC), adefovir dipivoxil (ADV), entecavir (ETV), telbivudine (TBH) and tenofovir-DF (TDF) would cause the extensive ranges of disadvantages on human beings after the consumption [4,5]. Therefore, the new strategies of potential anti-HBV drug are still needed. Accordingly, this research depends on natural sources for identifying novel drug to HBV.

Nature is the source of medicinal agents since time immemorial. Recently, the people are depending on treatment based on plant because of their fewer side effects. Currently, many bioactive molecules have been isolated from the medicinal plants and those are used by human beings for the purpose of making their life healthy. These bioactive compounds were isolated by various researchers, but it is needed to know about their molecular interactions. Nowadays, molecular docking mechanisms are adopted for model drug design to understand the drug and receptor binding interactions. The technology can help to design novel drug component. Therefore, the aim of the present study is to screen better drug candidate to inhibit the HBV complications through molecular docking.

RESULTS AND DISCUSSION

Crystal structure of Hepatitis B virus X protein (HBx)

Hepatitis B virus X protein (HBx) is a tiny authoritarian protein molecule that is conserved in mammalian hepadnaviruses [6]. Similar to HBx, the woodchuck hepatitis virus X protein (WHx) causes the viral infections to human beings and also binds DDB1 [7]. Bergametti *et al.* [8] have reported a partially conserved sequence motif in HBx and WHx that is essential to DDB1 involvements. The high resolution (2.9Å) HBx protein molecule was used for this molecular docking analysis with phytoconstituents.

Binding site analysis

In this study, the site analysis has shown five major active drugable binding sites from target molecule. Among the five sites, site 1 has been chosen for further study based on their site map score and volume of the area. The site map showed their binding cavity residues were TYR171, ALA221, ILE220, LYS168, TYR271, MET218, GLU65, ILE124, ILE123, VAL63, MET64, GLY122, ILE121, ILE120, GLY119, THR118, GLU312, ASN16, CYS313,

GLY17, LEU314, CYS18, VAL19, THR315, CYS260, HIS261, ASN262, ARG263, PRO223 and VAL222 (Figure 1). Likewise, Iftikhar *et al.* [9] have examined the ligand binding site from 4-aminobutyrate aminotransferase for docking with known analogues. Lots of computational studies have been not only applied to this research but also that carried out this binding site analysis techniques in various target molecules. Our previous research has found the active binding cavity in the target of PPAR γ [10–12]. As a result of this analysis, the qualified binding site was taken for grid generation, which fix the drugable binding site on target [13].

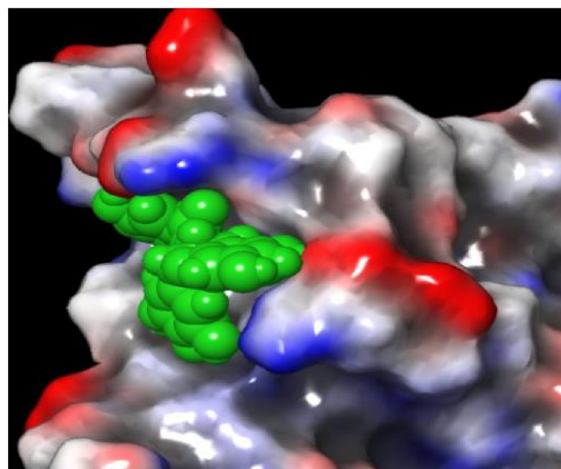


Figure 1. Ligand active binding cavity HBx.

Molecular docking

The molecular docking was carried out in the molecule of hepatitis B virus X protein (HBx) with experimentally reported and known phytoconstituents of medicinal plants. Among them, repandusinic acid is positioned superior docking score, with compared to other ligand molecules (Table 1). It also shows better binding affinities with a protein molecule. Among the 40 bioactive molecules, the repandusinic acid had a better docking score of -16.768 followed by corilagin, furosin, nirurin, iso-quercetin and galocatechin were also shown favorable docking scores of this study (Table 1). Through molecular docking we found that six bioactive molecules from the ligands have received the encouraging docking score with the target of human hepatitis B viral capsid. In this analysis, most of the molecules have exhibited excellent docking scores with the receptor molecule, which begins with the docking scores of -8.07 . Recently, lots of computational approach has been developed to find the effective drug molecules against worldwide challenging diseases and disease-causing organisms [14]. The

Table 1 Docking results of 40 natural compounds from medicinal plant sources

No.	Compound names	Name of the plant	Number of residues interactions	Glide docking XP score	Glide e-model (kcal/mol)
1	Reperandusinic acid	<i>Phyllanthus niruri</i> L.	Asn16, Glu312, Arg263, Lys168, Glu65, Ala62, Thr118	-16.180	-125.993
2	Corilagin	<i>Phyllanthus niruri</i> L. <i>Phyllanthus emblica</i> L.	His261, Arg263, Ala221, Cys18, Lys168, Gly122	-13.399	-92.836
3	Furosin	<i>Phyllanthus niruri</i> L.	Glu312, Cys18, Asn262, Arg263, Lys168 (salt bridge)	-13.454	-98.141
4	Nirurin	<i>Phyllanthus niruri</i> L.	Asn262, His261, Cys18, Thr118, Glu65, Tyr171	-12.392	-79.381
5	Iso-quercetin	<i>Hemidesmus indicus</i> L.	Cys18, Ile124, Glu65, Ala221, Arg263, His261 (salt bridge)	-12.299	-86.492
6	Rutin	<i>Phyllanthus niruri</i> L. <i>Delonix elata</i> L.	Glu65, Ile124, Cys18, Thr20, Ala221, Arg263, His261(salt bridge)	-11.848	-88.335
7	(+)Galocatechin	<i>Phyllanthus niruri</i> L.	His261, Thr118, Lys168, Asn16	-11.216	-95.126
8	Hesperidin	<i>Citrus</i> sp. L.	Ala221, Cys18, Thr20, Arg263, Leu66	-10.247	-115.29
9	Quercitrin	<i>Phyllanthus niruri</i> L. <i>Acalypha indica</i> L.	Leu314, Tyr171, Glu65, Ala262, Arg263, His261(salt bridge)	-10.009	-105.236
10	Astragalgin	<i>Phyllanthus niruri</i> L.	Ala221, Lys168, Arg263, Arg262, Glu65, Cys18	-9.503	-94.263
11	Geranin	<i>Phyllanthus niruri</i> L.	Arg263, His22, Thr20, Arg68, Leu66, Glu65, Cys18	-9.221	-97.741
12	Fisetin	<i>Mangifera indica</i> L.	Arg263, Ala221, Glu65, Cys18	-8.870	-82.586
13	Myricetin	<i>Holigarna grahamii</i> (Wight) Kurz.	Leu314, Glu65, Lys168, Glu65	-8.346	-82.077
14	Ascorbic acid	<i>Nervilia aragona</i> L.	Arg263, Tyr271, Ala221, Lys168, His261, Asn262	-8.304	-84.664
15	Astragalgin	<i>Cuscuta chinensis</i> L.	Leu66, Glu65, Ile124, Asn262, Phe169, Lys168	-8.254	-92.314
16	Kaempferol	<i>Jatropha curcas</i> , <i>Phyllanthus niruri</i> L.	Arg263, Ala221, Lys168, Glu168, Glu65, Leu314	-8.109	-98.9
17	Gallic acid	<i>Phyllanthus niruri</i> L. <i>Aerva lanata</i> L.	Arg263, Ala221, Lys168	-7.943	-97.417
18	Tartaric acid	<i>Gisekia phanaceides</i>	Arg263, Tyr271, His261, Lys168	-7.652	-75.934
19	Nirphyllin	<i>Phyllanthus niruri</i> L.	His261, Cys18, Gly122	-7.570	-90.786
20	Quinic acid	<i>Holigarna grahamii</i> (Wight) Kurz.	Asn16, Met66, Ile61	-7.481	-80.054
21	Chlorogenic acid	<i>Eryngium planum</i> L.	Asn262, Arg263, Tyr 271, Ile124	-7.238	-78.122
22	Caffeic acid	<i>Convolvulus gangeticus</i>	Arg263, Tyr271, Ile124	-7.143	-63.632
23	Isovitexin	<i>Jatropha mollissima</i> (pohl) baill	Glu312, Asn16, His261, Arg263, Ala221	-7.006	-68.914
24	(-) Epicatechin	<i>Camellia sinensis</i> L.	Thr20, Glu65, Lys168, Ala221, Arg263, His 261	-6.980	-91.758
25	(+) Catechin	<i>Camellia sinensis</i> L. <i>Albizia lebbeck</i> L.	His261, Leu314, Glu65, Met64, Ile61	-6.650	-72.007
26	Eriodictyol	<i>Lyonia ovalifolia</i> L. <i>Lythrum salicaria</i> L.	Thyr20, Thr315, His261, Arg263, Ala221	-6.531	-85.558
27	Ferulic acid	<i>Beta vulgaris</i> L.	Tyr271, Arg263, Ile124	-6.428	-81.391
28	Shikonin	<i>Lithospermum erythrorhizon</i> Siebold & Zucc	Aerg263, Phe169, Ile124	-6.221	-86.663
29	Venellic acid	<i>Capparis zeylanica</i> L.	Asn262, Arg263, Tyr271, Lys168	-6.118	-70.845

(Continued)

No.	Compound names	Name of the plant	Number of residues interactions	Glide docking XP score	Glide e-model (kcal/mol)
30	Estrodiol	<i>Momordica charantia</i> L.	Arg263, Phe169, Ile124	-6.007	-69.064
31	Taraxacin	<i>Calotropis procera</i>	Tyr271, Arg263, Ile124	-5.743	-37.129
32	Brevifoin	<i>Phyllanthus</i> sps.	Arg263, Ala221, Lys168	-5.520	-65.546
33	Phyltetralin	<i>Pilates niruri</i> L.	Cys18	-5.517	-58.561
34	(-) Limonine	<i>Limonia acidissima</i> L.	Cys18 Thr20, Leu314, Gly122	-5.481	-69.007
35	Hypophyllanthin	<i>Phyllanthus niruri</i> L. <i>Phyllanthus amarus</i> L.	Arg263, Asn262, Lys168, Ile124	-5.372	-71.809
36	Taraxastrol	<i>Calotropis procera</i>	Ile165	-5.204	-57.032
37	Niranthin	<i>Phyllanthus niruri</i> L.	Thr20, Cys18, His261	-5.029	-54.594
38	Salicylic acid	<i>Filipendula ulmoria</i> L.	Lys168, Ile124	-4.858	-73.773
39	Linolenic acid	<i>Nervilia aragona</i> L.	Tyr271, Arg263	-4.629	-76.13
40	Tridecanol	<i>Nervilia aragona</i> L.	Tyr271, Ala221	-4.568	-51.578

computational analysis provide us various essential particulars like docking score, binding energy, binding affinities and also show the ligand potentiality. Generally, the binding affinities were shown predominantly the ligand involvement and its flexibility with the protein cavity residues. In this complex structure, we identified such kinds of docking parameters and binding affinities that contact with ligands to target, which are essentially occupied in the hydrogen bond side chain, back bone and π - π stacking contacts.

Repandusinic acid

The small molecule of repandusinic acid had the effective docking scores of -14.768 and docking parameters such as ligand docking scores. The energy values were displayed in Table 1. It has revealed well countable binding affinities with the residues of human hepatitis B

viral protein. Here, many binding contacts were involved with target residues. Some of the interactions depend on both specific interactions with the ligand binding site and non-specific forces to the outside of the target binding cavity. Various kinds of binding affinities contributed to ligand and target. Exactly, hydrogen bond side chain, back chain and Pi-Pi stacking contacts were occupied between both molecules. After careful examination of the ligand and protein complexes, their contact arrangement was found. The complex displayed nine binding contacts between those two molecules (target to ligand). The residues GLU65, ALA62, THR118, GLU312, ASN16, LEU314, ARG63, ALA221 and LYS168 contacts with repandusinic acid which are displayed with their hydrogen distances in Figure 2. From this observation, we found ARG63 covalently binds with that of small molecule which connects back bone and salt bridge contacts. Among the residues contacts, eight residues

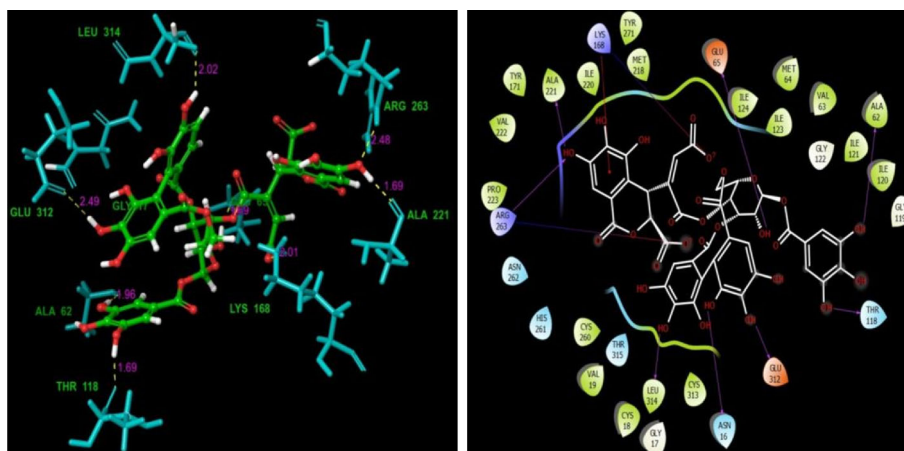


Figure 2. Residues and hydrogen bond contacts (yellow dotted line) with their distance values (pink) in repandusinic acid, and the 2D template representing the types of contacts involved between the ligand and target.

contacts belong to hydrogen bond back bone such as GLU65, ALA62, THR118, GLU312, ASN16, LEU314 and ALA221, which are all binds with ligand hydroxyl groups (OH) (Figure 2). LYS168 also connects a salt bridge and hydration site contacts with ligand oxygen groups. Here, the hydrogen bond side chain is indicated by the dotted blue straight line. The hydrogen bond back bone is indicated by solid blue straight line. And, the dumbbell shaped like a green color line indicated the Pi-Pi stacking contact. The red line indicated the hydration site contact. The repandunic acid is an alkaloids nature of the phytoconstituent which are available in *Phyllanthus amarus* medicinal plant. It is was confirmed to wide range of bio potential for many disease complications, which includes radical scavenging activity and can be a drug for Anti viral activity especially Virus like HIV-1 (protease) (Raintree Nutrition, Inc. Carson City, Nevada 89701, 2004).

Corilagin

Among the 40 small molecules, corilagin had the next docking scores of this study (-12.921) (Table 1). Examination of the docked revealed that the residue contacts include CYS18, LYS168, GLY122, VAL259, HIS261, ARG263 and ALA221. Figure 3 shows the residue contacts in dotted yellow and their distance values that were represented in pink. The hydrogen bond distance values were 1.92 (VAL259), 2.52 (CYS18), 1.98 (HIS261), 2.78 (ARG263), 1.86 and 1.95 (ALA221), 2.34 (LYS168) and 1.69 and 1.84 (GLY122), respectively. In interaction map, all the residues were involved in formation of hydrogen bond back bone contacts with ligand. Specifically, the residue LYS168 was formed of both hydrogen and hydration site contacts with ligand oxygen groups. While, the residue ARG263 was involved in hydrogen back chain contacts and hydration site with ligand hydroxyl group. Surprisingly, the residue GLY122 is involved in covalent hydrogen bond back bone

formation with corilagin. Corilagin is one of the important phytpconstituent in *Phyllanthus niruri* [16]. It is present in the nature of the ellagitannin. This molecule can also be found in other plants such as *Alchornea glandulosa*, *Caesalpinia coriaria* and *Punica granatum*. The extensive pharmacological activities of corilagin have been articulated, such as anti-atherogenic, anti-oxidant, hepatoprotective, and anti-tumor [17–20]. This shows the favorable docking score with the human hepatitis B viral protein (HBx) molecule (Table 2).

Furosin

Furosin had the third valuable docking score -12.58 (Table 1). Investigation of the docked complex demonstrates that the contacts include LYS168, GLY122, ALA62, GLU312, CYS18, ASN262, HIS261 and ARG263. In the molecular structure, the residues has shown that their contacts distance values were 2.18 (LYS168), and covalent contacts were 1.87 and 1.80, 1.93, 2.11, 1.82, 2.23 and 1.69, respectively. The residue contacts and their distance values were highlighted in pink color in Figure 4. From this interaction map, we found that the residues contacts with various atoms of the ligand molecule. Of them, the residues LYS168 and ASN262 were both involved in hydrogen bond back bone formations with the oxygen groups of furosin. Also, the residues GLY122, ALA62, GLU312, CYS18 and HIS261 were all involved in hydrogen bond back bone contacts with the hydroxyl groups of small molecule. But the residue ARG263 binds with ligand by the arrangement of salt bridge. Figure 4 has shown their binding contact types. Previously, the bioactive molecule of furosin were reported in various medicinal plants such as *Phyllanthus emblica*, *P. virgatus*, *P. sellowianus* and *P. debilis*. These plants have diverse biological activities including anti-oxidant, anti-hyperalgesic and wound healing [21–25]. Nevertheless, this molecular docking approach has presented their potentials against the hepatitis B virus.

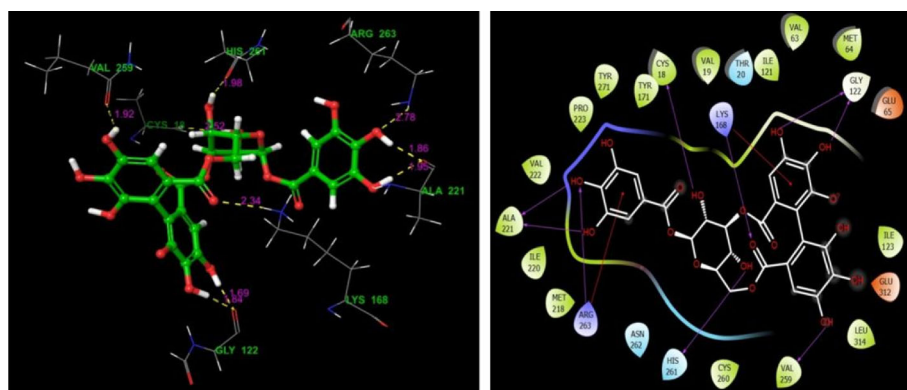


Figure 3. Residues and hydrogen bond contacts (yellow dotted line) with their distance values (pink) in corilagin, and the 2D template representing the types of contacts involved between the ligand target.

Table 2 Physicochemical properties and biological functions of the 40 bioactive molecules analyzed by using QuikProp

No.	Molecular formula	Molecular weight (g/mol)	Volume	SASA	Acceptor H bond groups	Donor H bond groups	Number of ring atoms	Q Plog Pw (-2 to 6.5)	% Human oral absorption	CNS	Rule of five
1	C ₄₁ H ₃₀ O ₂₈	970.663	2327.586	1050.003	17	29.95	55	-2.557	1	-2	3
2	C ₂₇ H ₂₂ O ₁₈	634.455	1552.483	778.079	9	20.55	28	-1.354	1	-2	3
3	C ₂₇ H ₂₂ O ₁₉	650.454	1671.132	866.924	7	20.05	28	-3.007	1	-2	3
4	C ₃₂ H ₄₀ O ₁₅	664.651	1568.806	821.477	7	22.45	17	-1.397	1	-2	3
5	C ₂₁ H ₂₀ O ₁₂	464.379	1611.303	837.282	7	19.30	28	1.884	1	-2	3
6	C ₂₇ H ₃₀ O ₁₆	610.521	2190.041	1057.957	6	21.30	34	0.453	1	-2	3
7	C ₁₅ H ₁₄ O ₇	306.270	1519.296	807.567	10	10.90	32	-0.515	1	-2	3
8	C ₂₈ H ₃₄ O ₁₅	610.565	995.503	553.076	6	9.65	12	-2.426	1	-2	1
9	C ₂₁ H ₂₀ O ₁₁	448.380	963.976	535.187	9	12.35	6	-1.76	1	-2	2
10	C ₂₁ H ₂₀ O ₁₁	448.380	1127.265	634.363	7	13	20	0.957	1	-2	2
11	C ₄₁ H ₂₈ O ₂₇	952.648	1094.899	637.616	5	7	12	2.223	1	-2	0
12	C ₁₅ H ₁₀ O ₆	286.239	1443.524	746.044	6	9.35	17	0.427	2	-2	1
13	C ₁₅ H ₁₀ O ₈	318.237	856.481	503.931	5	5.45	16	-3.079	2	-2	0
14	C ₆ H ₈ O ₆	176.124	595.353	369.932	6	10.20	0	-1.245	1	-2	1
15	C ₂₁ H ₂₀ O ₁₁	448.380	794.879	466.636	5	12.40	6	3.323	1	-2	0
16	C ₁₅ H ₁₀ O ₆	286.239	1093.897	616.277	2	4	16	0.286	3	-2	0
17	C ₇ H ₆ O ₅	170.120	834.190	477.400	5	5.45	16	1.289	2	-2	0
18	C ₄ H ₆ O ₆	150.086	1041.592	547.798	3	8.10	15	-0.359	3	-2	0
19	C ₂₄ H ₃₂ O ₈	448.512	836.626	516.586	3	8.60	11	3.561	2	-2	0
20	C ₇ H ₁₂ O ₆	192.167	1294.790	378.032	17	29.95	55	-2.557	1	-2	3
21	C ₁₆ H ₁₈ O ₉	354.311	868.249	578.065	9	20.55	28	-1.354	1	-2	3
22	C ₉ H ₈ O ₄	180.159	831.747	436.980	7	20.05	28	-3.007	1	-2	3
23	C ₂₁ H ₂₀ O ₁₀	432.381	878.822	578.603	7	22.45	17	-1.397	1	-2	3
24	C ₁₅ H ₁₄ O ₆	290.271	870.576	473.047	7	19.30	28	1.884	1	-2	3
25	C ₁₁ H ₁₂ O ₆	280.712	553.076	668.294	6	21.30	34	0.453	1	-2	3
26	C ₁₅ H ₁₂ O ₆	288.255	525.074	621.747	10	10.90	32	-0.515	1	-2	3
27	C ₁₀ H ₁₀ O ₄	194.186	643.360	873.822	6	9.65	12	-2.426	1	-2	1
28	C ₁₆ H ₁₆ O ₅	288.299	746.044	975.780	9	12.35	6	-1.76	1	-2	2
29	C ₈ H ₈ O ₄	168.148	396.249	780.638	7	13	20	0.957	1	-2	2
30	C ₁₈ H ₂₄ O ₂	272.388	661.231	535.750	5	7	55	2.223	1	-2	3
31	C ₁₅ H ₁₄ O ₃	242.274	456.890	535.178	6	9.35	28	0.427	2	-2	3
32	C ₁₅ H ₁₂ O ₆	288.255	474.403	643.336	5	5.45	28	-3.079	2	-2	3
33	C ₁₀ H ₁₀ O ₄	194.186	574.897	673.616	6	10.20	17	-1.245	1	-2	3
34	C ₁₆ H ₁₆ O ₅	288.299	561.568	530.931	5	12.40	28	3.323	1	-2	3
35	C ₈ H ₈ O ₄	168.148	578.158	661.271	2	4	34	0.286	3	-2	3
36	C ₁₈ H ₂₄ O ₂	272.388	562.372	464.663	5	5.45	32	1.289	2	-2	3
37	C ₂₄ H ₃₂ O ₇	432.513	673.661	474.700	3	8.1	12	-0.359	3	-2	1
38	C ₁₂ H ₁₆ O ₃	208.257	530.913	574.879	3	8.6	6	3.561	2	-2	2
39	C ₁₈ H ₃₀ O ₂	278.436	396.932	578.851	17	29.95	20	-2.557	1	-2	2
40	C ₁₃ H ₂₈ O	200.366	526.723	562.372	9	20.55	12	-1.354	1	-2	0

SASA, solvent accessible surface area; CNS, central nervous system

valuable docking scores of -11.836 , which showed good binding affinities with target (Table 1). Determination of this docked complex showed their binding affinities, such as H-bond side chain, back chain, π - π stacking and salt bridge contacts. Iso-quercetin interacts with the target residues including ARG263, ALA221, GLU65, ILE124, CYS18 and HIS 261 respectively (Figure 6). For this predicted complex molecule, binding affinities was shown by means of the route of interaction map. It displayed two kinds of contacts lines like hydrogen bond backbone and π - π stacking contacts. Specifically, the residues ARG263 and ALA221 formed ionic bond contacts with functional groups. CYS 4 was displayed in blue solid aero line with contacts on ligand hydroxide group (Figure 6). Iso-quercetin is a flavonoids nature of phytoconstituents which is frequently obtained from *Phyllanthus emblica*, *P. urinaria*, *P. reticulatus*, *P. virgatus* and *P.muellerianus* [37]. These plants were also involved in diverse range of biological actions.

ADME analysis

In this computational investigation, the physicochemical and biological properties of 40 phytoconstituents were listed in table 2. This study reveals the properties of known phytoconstituents such as molecular weight, molecular formula, volume, solvent accessible surface area, H-bond acceptor, H-bond donor, ring atoms, Q Plog, human oral absorption and central nervous system [38]. In recent scenario, the ADME related studies have been greatly helpful for the development of better drugs to globally challenging diseases and disorders.

CONCLUSIONS AND FUTURE FOCUS

The present study focused on hepatitis B virus that infects human beings due to the virus replication. At present, the

conventional drugs of Hepatitis B virus induce side effects to human and few are not responsible for that diseases complication. Present computational studies found a few effective phyto-chemicals for hepatitis B virus which are identified based on their mode of interactions with hepatitis B viral protein molecule. Among the molecules, repandusinic acid has a better docking score and it was shown to good binding affinities with the target. Furthermore, the other phytochemicals such as corilagin, furosin, nirurin, iso-quercetin and gallicocatechin also get better docking scores. Hence, this study concluded that the phytochemicals are a better drug candidate for hepatitis B virus. *In vitro* and *in vivo* evaluation will be essential and we hope this computational result will be helpful to proceed further with the effective drug development.

MATERIALS AND METHODS

Software and hardware

Computational analysis was carried out in Maestro v10. 2. It was complexly programmed in single software which includes ligprep, sitemap, glide grid and glide Xtra Precision. This software was installed in DELL PRECISION T1700 workstation machine which is running on Intel (R) Core (TM) i5-4590 CPU processor with 8 GB RAM and 240 GB hard disk. Centos Linux was used as the operating system [39].

Biological data

The protein molecule of hepatitis B virus X protein (HBx) was retrieved from Protein data bank [40]. The target molecule data bank alpha numeric ID is 3I7H. Totally, 40 phytoconstituents were chosen for this docking investigation. These molecules were identified and selected with the help of previous research findings. Later, the identified

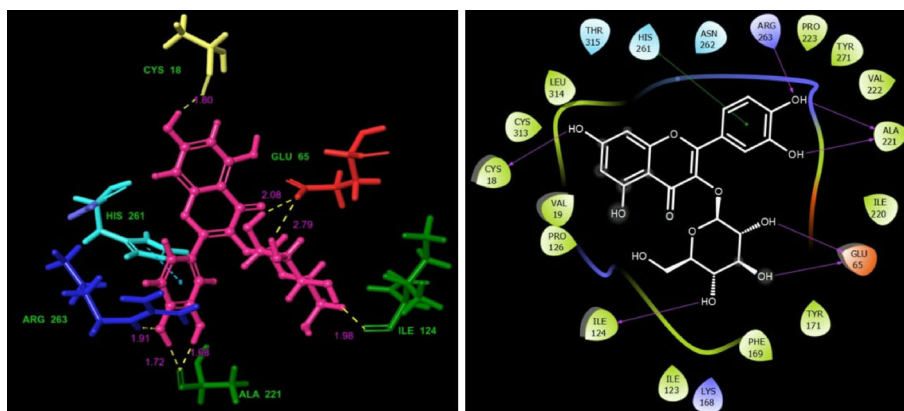


Figure 6. Residues and hydrogen bond contacts (yellow dotted line) with their distance values (pink) in iso-quercetin, and the 2D template representing the types of contacts involved in the ligand and target.

molecules were obtained from chemical database and some were drawn in Maestro v10.2 [41].

Protein preparation

Protein preparation were made by the protein preparation wizard tool. They resolved some target harms such as the missing of side chains and the adding of back bones, and the revising of the missing residues. The x-ray crystallography structure of the target is strictly coupled with the water molecules. The water molecules occupied by protein is not qualified to acquire the docking study. Hence, through this process, we evacuated the water molecules and increased the entropy of the target. The protein preparation was followed by the method of Prabhu *et al.* [42].

Active site validation and grid generation

The site validation was examined by the target inner and surface regions with the help of sitemap tool in Maestro v10.2 [43]. In the site analysis, the target showed possible active binding sites. Among them, one binding site was selected to further study based on their site values viz, site score and site area volume. Then, the selected site was used for the grid generation. Grid box shape and values were generated in $X: 5.3; Y: 3.71; Z: -20.86$ coordination. Target site was explained with 10 Å radius around the ligand binding site [44].

Ligand preparation

The structures of the 40 ligand molecules were converted to 3D structure by using ligprep tool [45]. The ligands were geometrically optimized via Optimized Potentials for Liquid Simulations 2005 (OPLS2005) force field [40]. LigPrep is used in Schrodinger suite for the purpose of generating ligand of 3D structures from 1D (Smiles) and 2D (SDF) representation, probing for tautomers and steric isomers and geometry minimization of ligands [46,47].

Molecular docking

The molecular docking study was used to perform Xtra precision docking mode in our study. It was used for predicting the binding affinities of target to ligand, ligand efficiency, and ligand inhibitory constant to the target. The complex of thirty nine ligands was docked with the active site of the target by using Glide Xtra precision (XP) which docks ligands [44,48]

ADMET analysis

This analysis was carried out by QuikProp tool in Masetro

v10.2. All the ligand molecules were analyzed with the parameters like hydrogen bond donor, hydrogen bond acceptor, blood brain barrier, central nervous system followed by Vijayakumar *et al.* [2], and Morvin *et al.* [49]

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COMPLIANCE WITH ETHICS GUIDELINES

The authors Vijayakumar Subramaniyan, Reetha Sekar, Arulmozhi Praveenkumar and Rajalakshmi Selvam declare that they have no conflict of interests.

All procedures performed in studies were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

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