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An *in-silico* study encompassing virtual screening and ADME/T properties of phytochemicals present in leaf extract of *Trapa natans* in relevance to sodium taurocholate co-transporting polypeptide involved in hepatitis B

Mudassir Alam, Kashif Abbas, Mohd Ali, Shakir Kamal and Shipra Bhagia

Abstract
Hepatitis B virus (HBV) is an essential human disease and hepatotropic virus. An estimated 296 million chronically infected persons in the globe, and many of them may acquire severe liver disorders such as hepatitis, cirrhosis, hepatocellular cancer (HCC) and hepatomegaly. *Trapa natans* L. thrives in lakes and rivers with sunny locations and slightly acidic water. According to the literature, water chestnut ae rich in crucial minerals, proteins, lipids, carbohydrates, vitamins, dietary fibres, polyphenols such as phenolic acids and flavonoids, and hydrolysable tannins. *Trapa natans* are rich in phenols and flavonoids. Several investigations have shown that *Trapa natans* leaf extract has potential pharmacological action, including hepatoprotective and anti-inflammatory properties. The study aims at *In silico* and ADME/T analysis of active compounds present in *Trapa natans* leaf extract against NTCP to evaluate and visualize protein-ligand interaction.

Keywords: *In silico*, hepatitis B, *Trapa natans*, NTCP, phytochemicals, modelling

1. Introduction
Hepatitis B is a potential global health scare as it is a fatal liver disease caused by hepatitis B virus (HBV). Persistent infection due to the disease may add to the count of debility and mortality from hepatocirrhosis and hepatocarcinoma [1]. According to WHO report, the disease occurrence in Western Pacific Region and the African Region seems to be contrasting than rest of the world. HBV's molecular machinery consist of a 3.2kb circular, partly double-stranded DNA molecule with four overlapping reading frames that code for surface proteins (HBsAg), core proteins (HBcAg/HBeAg), transcriptional trans activator X protein and the viral polymerase [2]. Protein such as preS1 domain present on the surface of the virus aid in its entry into hepatocytes [3]. Filamentous HBsAg subviral particles with substantial amount of the vast surface protein attach to hepatocellular membranes specifically, while spherical HBsAg subviral particles with a relatively low level of the large surface protein attribute to the membranes to a lesser degree [4]. Heparan sulfate proteoglycans (HSF), which are found on surface of cell, were observed to initiate and promote attachment of HBV to hepatocytes via low-affinity binding to the antigenic loop of the S protein [5]. Studies have shown that sodium taurocholate cotransporting polypeptide (NTCP) are vital HBV receptor [6].
NTCP is explicitly expressed on hepatocytes and its silencing suppresses progression of HBV infection [7]. Thus, NTCP may act as a therapeutic target for hepatitis treatment. *Trapa natans L.*, often known as water chestnut, is an annual aquatic floating plant endemic to Asia, where it is known by the local name Singhara. Water chestnut has long been utilised for medicinal reasons in the Asian continent, particularly India and China. *Trapa natans* is one of the most important medicinal plants in Indian Ayurveda because it treats stomach illnesses, genitourinary system disorders, and liver, kidney, and spleen issues [8]. *Trapa natans* have been discovered to be high in phenolics and flavonoids. Table 1 lists the active phenolic and flavonoids found in the leaf extract [9]. According to the literature, *Trapa natans* leaf extract has hepatoprotective, anti-inflammatory, anti-microbial, and anti-diabetic effects [10]. In silico tools are widely used nowadays in order to study protein-ligand interaction which could be further used for drug designing and drug development planning.

The study aims at virtual screening and pharmacokinetics of the active compound present in leaf extract of *Trapa natans* against NTCP in order to find favourable drug candidate for treatment of hepatitis.

Table 1: Phenolics and flavonoids present in *Trapa natans*

Flavonoids	Phenolics
Quercetin	Caffeic acid
Rhamnetin	p-Coumaric acid
Pinobanksin	Ellagic acid
Naringenin	Ferulic acid

2. Material and Methods

In-silico based study was performed using Hewlett Packard laptop with hardware configuration 8 GB RAM, Intel i3 11th generation. The molecular docking tool used for the *in-silico* study was AutoDock Vina tool [11] in PyRx [12]. PyMOL 2.4.1 [13] was used for the purpose of protein preparation and visualisation of the docked ligand with protein. Ligplot+ was used to analyse protein-ligand interaction [14]. For the purpose of ADME/T studies, SwissADME webserver was used [15]. In order to predict 3D protein structure and homology modelling, SwissModel webserver was used while using protein sequence retrieved from Uniprot database [16].

2.1 Retrieval and preparation of ligand

The active chemical compounds present in leaf extract of *Trapa natans* were accessed from the PubChem database (https://pubchem.ncbi.nlm.nih.gov/) in SDF file format. A total of 8 active compounds were retrieved and their detail is mentioned in Table 2. Ligand preparation was done using PyRx software. The energy of the ligands was minimised using Open Babel tool [17] within the PyRx software and force field used for energy minimisation was MMFF94. Finally, all the ligands were converted to Auto Dock pdbqt format.

Table 2: Phytochemicals with their related information which are used as ligand.

S. No.	Ligand name	Pubchem ID	Molecular weight	Molecular formula
1	Ferulic acid	445858	194.18	C10H10O4
2	Quercetin	5280343	302.23	C15H10O7
3	Rhamnetin	5281691	316.26	C16H12O7
4	Ellagic acid	5281855	302.19	C14H6O8
5	p-coumaric acid	637542	164.16	C9H8O3
6	Caffeic Acid	689043	180.16	C9H8O4
7	Pinobanksin	73202	272.25	C15H12O5
8	Naringenin	932	272.25	C15H12O5

2.2 Protein Structure prediction and validation

The NTCP sequence obtained from the Uniprot server (Entry number: Q14973) were utilised as protein query sequences for modelling. SWISS-MODEL (http:// swissmodel.expasy.org) was employed to estimate the three-dimensional structure of NTCP. Various approaches were utilised to assess the interior uniformity and steadiness of the NTCP-modelled structure. The PROCHECK [18] and MolProbity [19] programmes were used to evaluate the stereo-chemical quality of predicted model by measuring the residues in the Ramachandran plot's authorised zones. With help of QMean-Z-Scores (http://swissmodel.expasy.org/docs/structure assessment) [20], predicted protein structure was re-evaluated for its quality and reliability.

2.3 Virtual screening of the ligands

AutoDock Vina tool within PyRx software was used for the purpose of virtual screening of the retrieved active compounds against the desired target protein. Blind docking was performed with grid box dimension (47.05 Å ×47.46 Å ×62.97 Å) and centre (103.60, 107.62, 109.55). The exhaustiveness was set to 8 by default. After the completion of screening analysis, the candidates with good docking score were visualised using PyMOL.

2.4 Protein-ligand interactions

Ligplot+ software was used to make Ligplot for the prediction of hydrophobic and hydrogen bond interaction between the ligand and the target protein.

2.5 ADME/T analysis

The ADME/T analysis offers information on the examined ligand's or chemical compound's absorption, distribution, metabolism, excretion, and toxicity. The analysis excludes undesirable compounds with minimal medication similarity to make the research viable and time-efficient. With the SwissADME website (http://www.swissadme.ch/), the canonical SMILES of the retrieved compounds or hits were utilised to analyse the pharmacological and physiochemical profiles of the hit compound. For pharmacological validation, the following factors were considered: molecular weight (MW), number of hydrogen bond acceptors (Accept H), number of hydrogen bond donors (Donor H), GI absorbability, topological polar surface area (TPSA) value, and LogS value. SWISSADME's BOILED-Egg technology was used to analyse and forecast GI absorption and brain penetration of pharmacological compounds.

3. Result

3.1 Structure prediction and validation

Homological modelling of the NTCP was done using the simulated protein modelling server; SWISS-MODEL powered by ProMod3, which is an open structure comparative modelling engine (Figure1). The NTCP model was evaluated using the Ramachandran plot from the PROCHECK program and verified all the amino acid residues of the modelled protein fit in the allowed regions of the Ramachandran plot. NTCP had a MolProbity score of 0.96, 97.63% of the residues were in the favoured regions, 0.34% were in the outliers' regions, and the Clash score was 0.00. The structure's QMEAN Z-score was reported to be -3.14. (Figure 2). The QMEAN Z-score offered an indication of the "degree of nativeness" of the structural characteristics identified in the model on a global scale. It reflects if the model's QMEAN score is equivalent to the predicted score from experimental structures of comparable size. A QMEAN Z-score value close to 0 indicates greater quality between the modelled and experimental structures. Scores of 4.0 or lower imply that the models are of poor quality. The NTCP's QMEAN Z-scores were -3.14, indicating that the suggested homology model is credible and acceptable. PROCHECK was used to verify the stereochemical quality of a protein structure by evaluating residue-by-residue geometry and overall structure geometry on the UCLA SAVES 6.0 server. Detail information related to PROCHECK is given in Figure (3 a-d). The stereochemical properties of the modelled structure is given in Table 3. Overall PROCHECK report is given in figure 4.

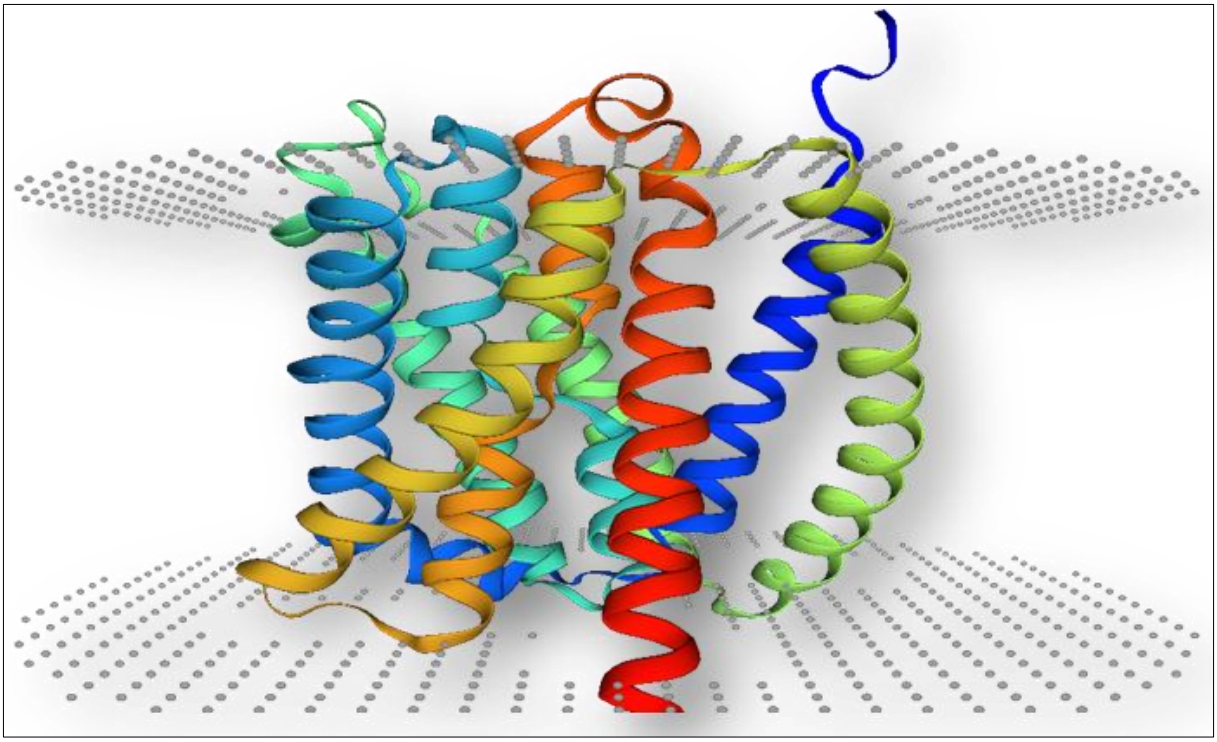


Fig 1: Modelled structure of NTCP

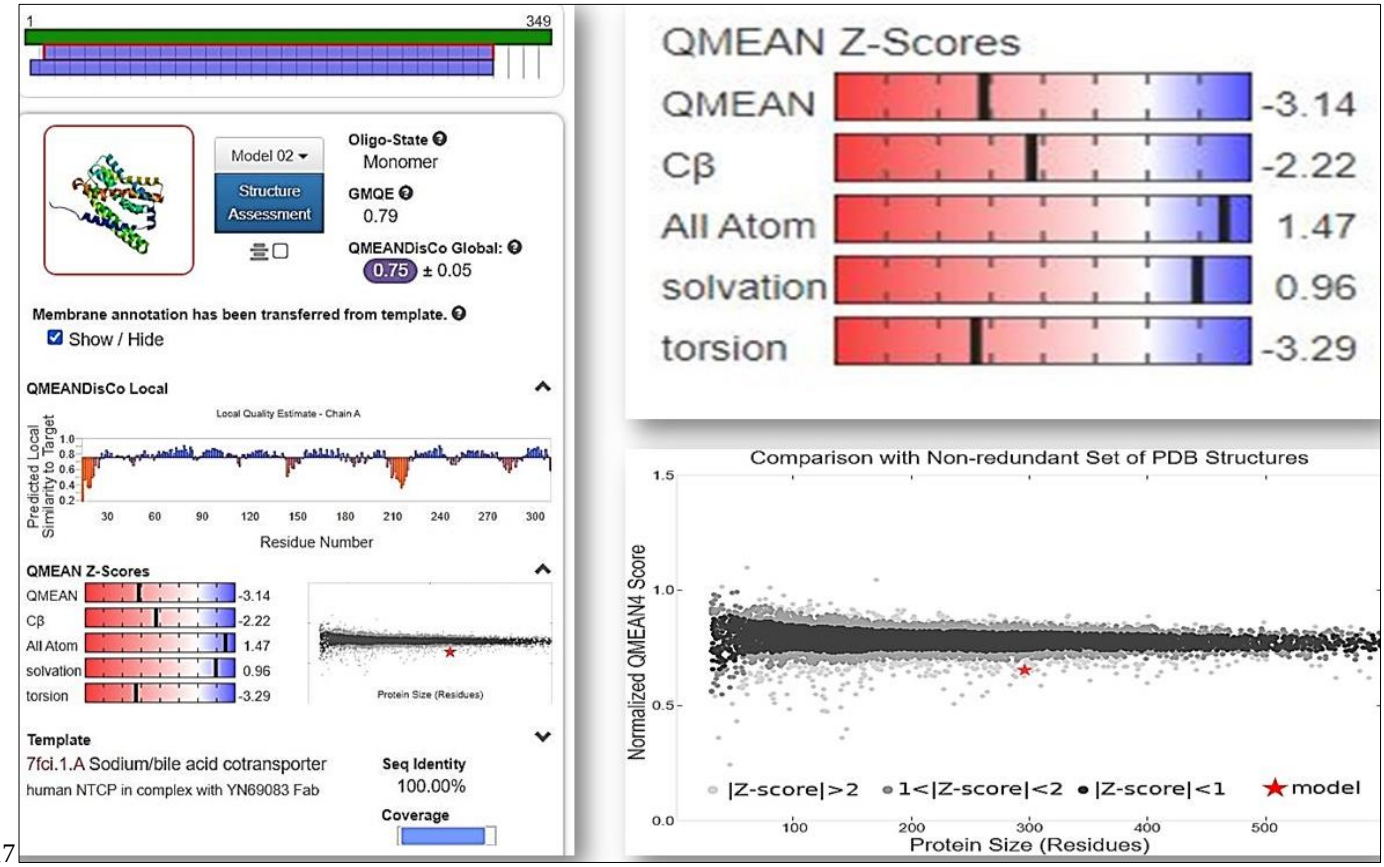
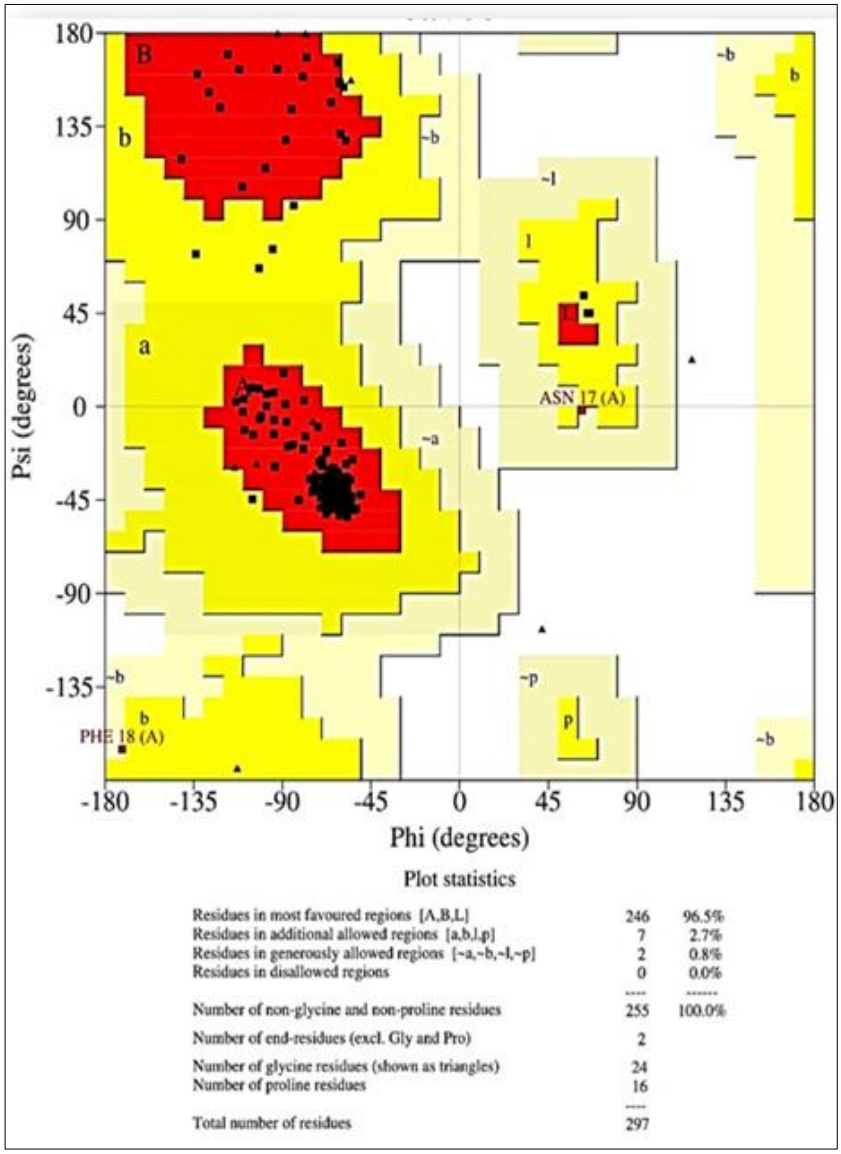
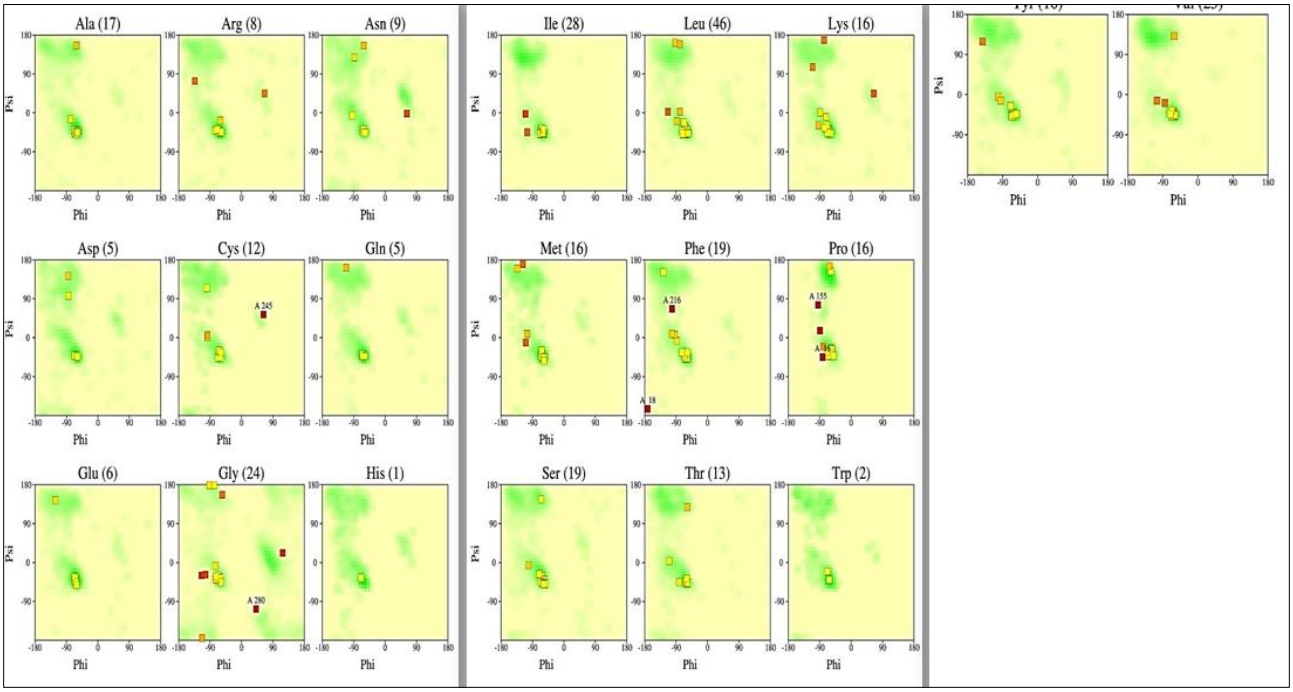


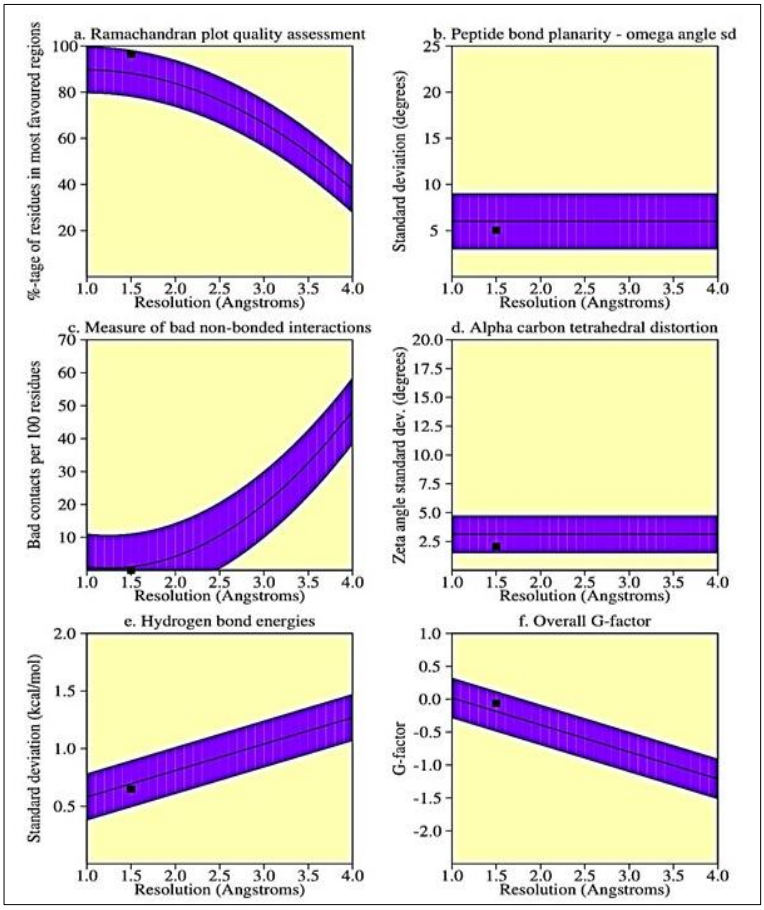
Fig 2: QMEAN Z-score calculated using structure assessment tool of SWISS-MODEL


Psi (degrees)
Phi (degrees)
ASN 17 (A)
PHE 18 (A)
Plot statistics
Residues in most favoured regions [A,B,L] 246 96.5%
Residues in additional allowed regions [a,b,l,p] 7 2.7%
Residues in generously allowed regions [~a,~b,~l,~p] 2 0.8%
Residues in disallowed regions 0 0.0%
Number of non-glycine and non-proline residues 255 100.0%
Number of end-residues (excl. Gly and Pro) 2
Number of glycine residues (shown as triangles) 24
Number of proline residues 16
Total number of residues 297

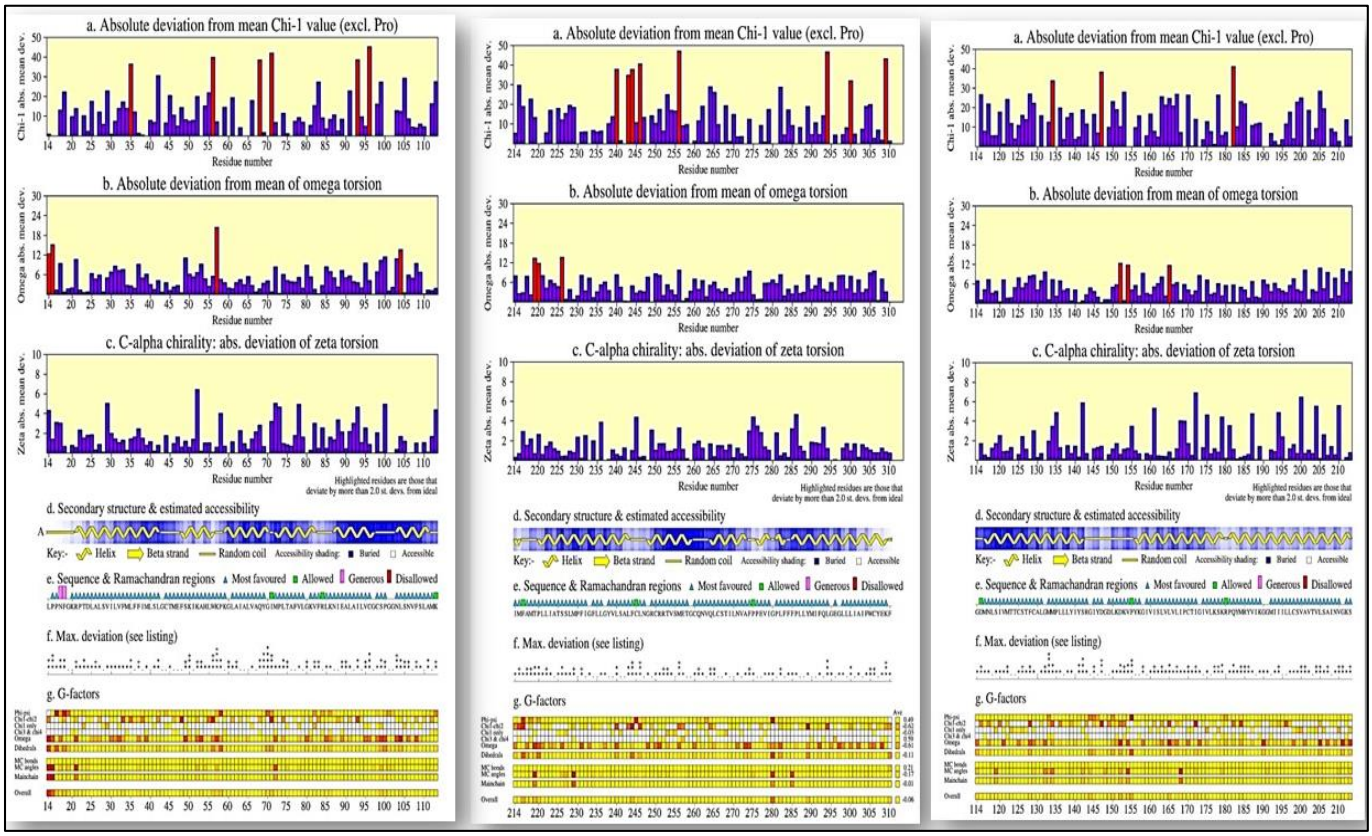
(a)


Ala (17)
Arg (8)
Asn (9)
Asp (5)
Cys (12)
Gln (5)
Glu (6)
Gly (24)
His (1)
Ile (28)
Leu (46)
Lys (16)
Met (16)
Phe (19)
Pro (16)
Ser (19)
Thr (13)
Trp (2)
Psi
Phi

(b)



(c)



(d)

Fig 3: (a) Ramachandran plot analysis: A decent quality model would be predicted to have over 90% in the most preferred area based on structural resolution analysis of at least 2.0 Angstrom and R-factor more than 20%. (b) Ramachandran plot for all residue type: Residual count are depicted in square boxes, and those in unfavourable conformations (< -3.00) are labelled. Shaded area implies favourable conformations as per analysis at 2 Angstrom resolution (c) Main chain parameters (details are given in Table 3) (d) Residue properties

Table 3: Stereochemical properties of predicted structure of NTCP

Stereo-chemical parameter	No. of data points	Parameter value	Typical Value	Bandwidth
Percentage residues in A,B,L	255	96.5	88.2	10
Omega angle SD	296	5	6	3
Bad contacts/100 residues	0	0	1	10
Zeta angle SD	273	2.1	3.1	1.6
H-bond energy SD	229	0.6	0.7	0.2
Overall G-factor	297	-0.1	-0.2	0.3

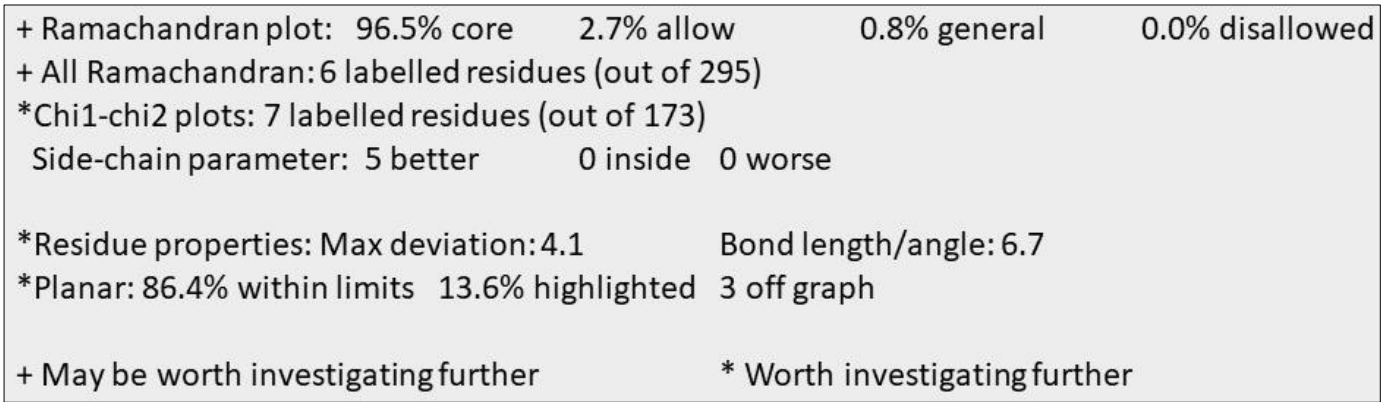


Fig 4: PROCHEK overall report obtained through UCLA SAVES 6.0 server

3.2 Virtual screening result Analysis

The three-dimensional crystal structure of NTCP obtained through homology modelling was used for virtual screening purpose. The 8 active compounds of *Trapa natans* leaf extract were retrieved from the PubChem database in Structural Data Format (SDF). The hit ligands or active compounds were screened against the desired target NTCP using the AutoDock Vina tool of PyRx. The docking score (kcal/mol) of the phytochemicals is given in Table 4.

Table 4: Docking score obtained after virtual screening of phytochemicals against NTCP

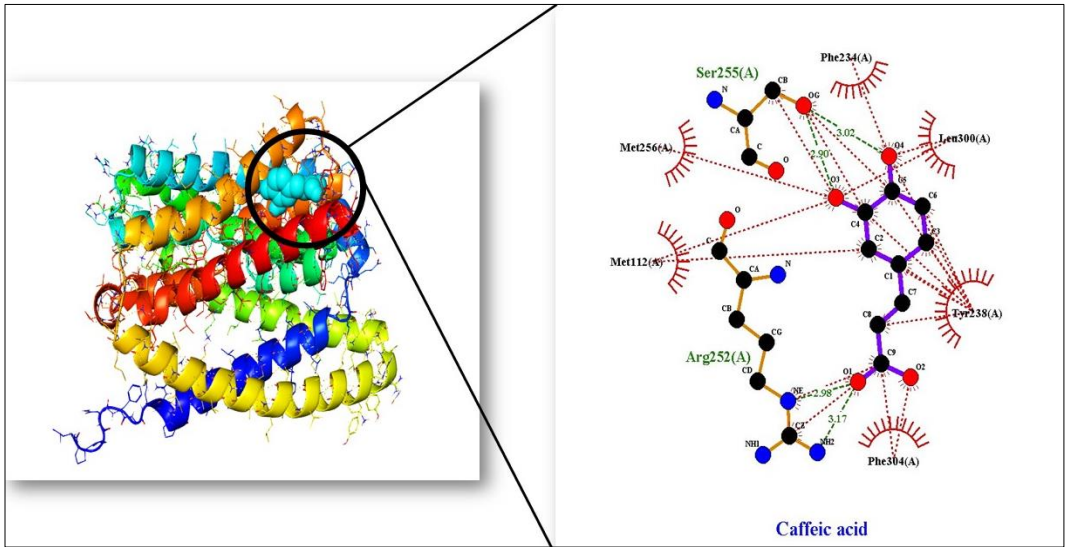
S. No.	Ligand Name	Pubchem ID	Molecular Weight	Molecular Formula	Docking Score (Kcal/Mol)
1	Ferulic acid	445858	194.18	C10H10O4	-5.3
2	Quercetin	5280343	302.23	C15H10O7	-7.1
3	Rhamnetin	5281691	316.26	C16H12O7	-7.2
4	Ellagic acid	5281855	302.19	C14H6O8	-7.3
5	p-coumaric acid	637542	164.16	C9H8O3	-5.5
6	Caffeic Acid	689043	180.16	C9H8O4	-5.7
7	Pinobanksin	73202	272.25	C15H12O5	-7.1
8	Naringenin	932	272.25	C15H12O5	-7.0

3.3 Protein-ligand interaction analysis

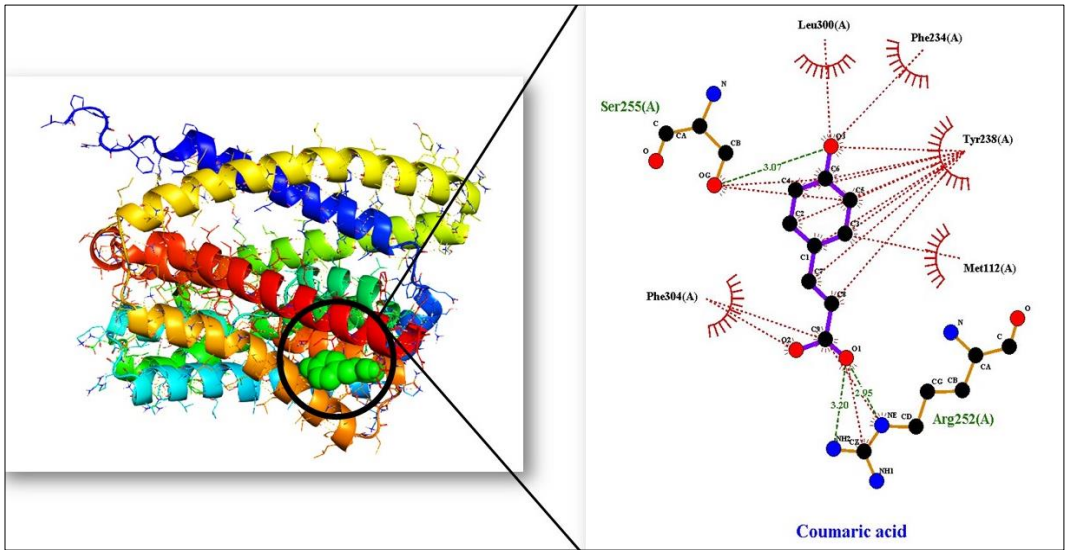
Ligplot+ software for protein-ligand has been used for analysis and highlighted the hydrogen bonds and hydrophobic interaction between the amino acids of the protein and the ligand. The analysis aid in analysing the binding affinity between the phytochemical and the target protein. Different types of interactions exhibited by the selected compounds is given in Table 5. The schematic 2D representation of interaction between the ligands or phytochemicals and the target protein is given in Figure 5.

Table 5: Interaction of phytochemical compound with active site of the target protein

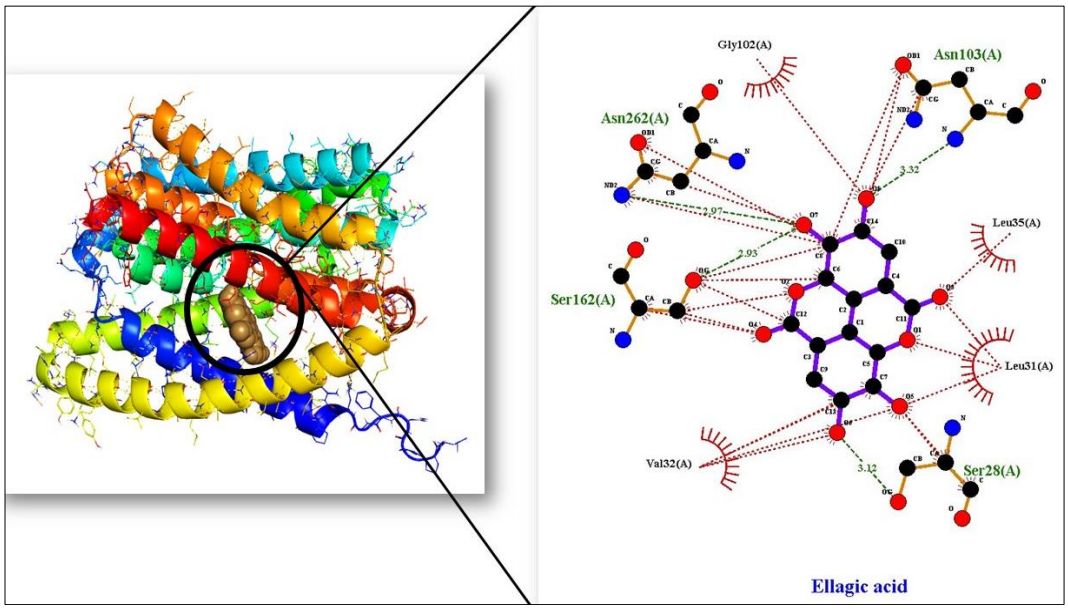
S. No.	Ligand Name	Pubchem ID	Hydrophobic Interaction	Hydrogen Bond Interaction	Docking Score
1	Ferulic acid	445858	Met112, Phe234, Leu235, Tyr238, Phe304	Arg252, Ser255	-5.3
2	Quercetin	5280343	Leu27, Leu31, Val202, Ser206, Asn209, Phe284, Phe286, Met290	Gln264, Phe283, Leu284	-7.1
3	Rhamnetin	5281691	Leu27, Leu31, Val202, Ser206, Asn209, Phe284, Pro286, Met290	Gln264, Phe283, Leu287	-7.2
4	Ellagic acid	5281855	Leu31, Val32, Leu35, Gly102	Ser28, Asn103, Ser162, Asn262	-7.3
5	p-coumaric acid	637542	Met112, Phe234, Tyr238, Leu300, Phe304	Arg252, Ser255	-5.5
6	Caffeic Acid	689043	Met112, Phe234, Tyr238, Met256, Leu300, Phe304	Arg252, Ser255	-5.7
7	Pinobanksin	73202	Phe216, Gly280, Phe283, Phe284	Lys20, Lys212, Pro276	-7.1
8	Naringenin	932	Phe18, Pro276, Ile279, Phe283	Lys20, Asn271,Gly280	-7.0


Ser255(A)
Phe234(A)
Leu300(A)
Met256(A)
Met112(A)
Tyr238(A)
Arg252(A)
Phe304(A)
Caffeic acid

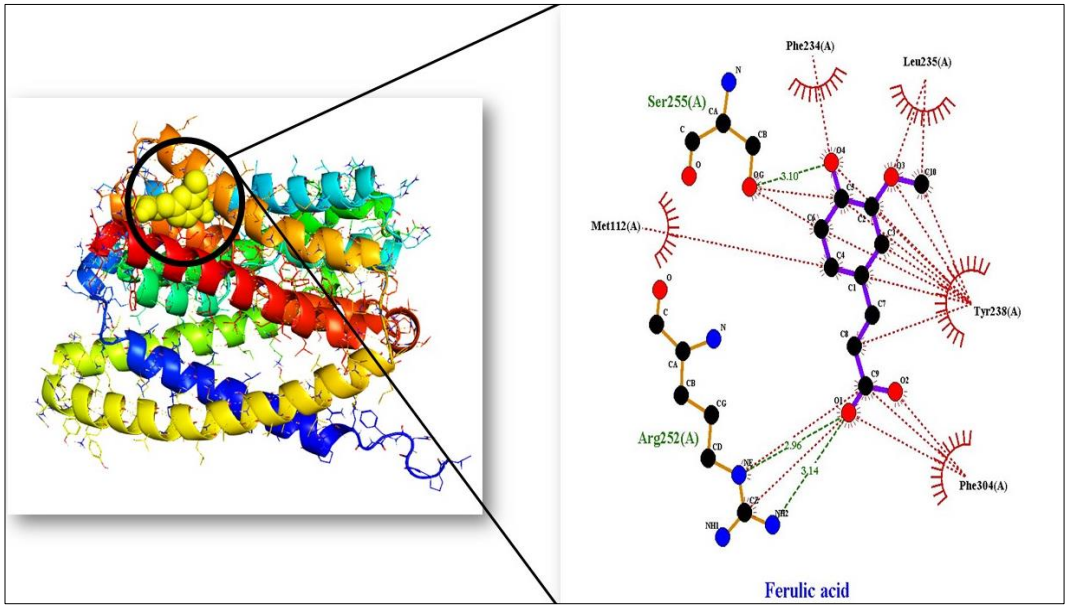
(a)


Leu300(A)
Phe234(A)
Ser255(A)
Tyr238(A)
Met112(A)
Phe304(A)
Arg252(A)
Coumaric acid

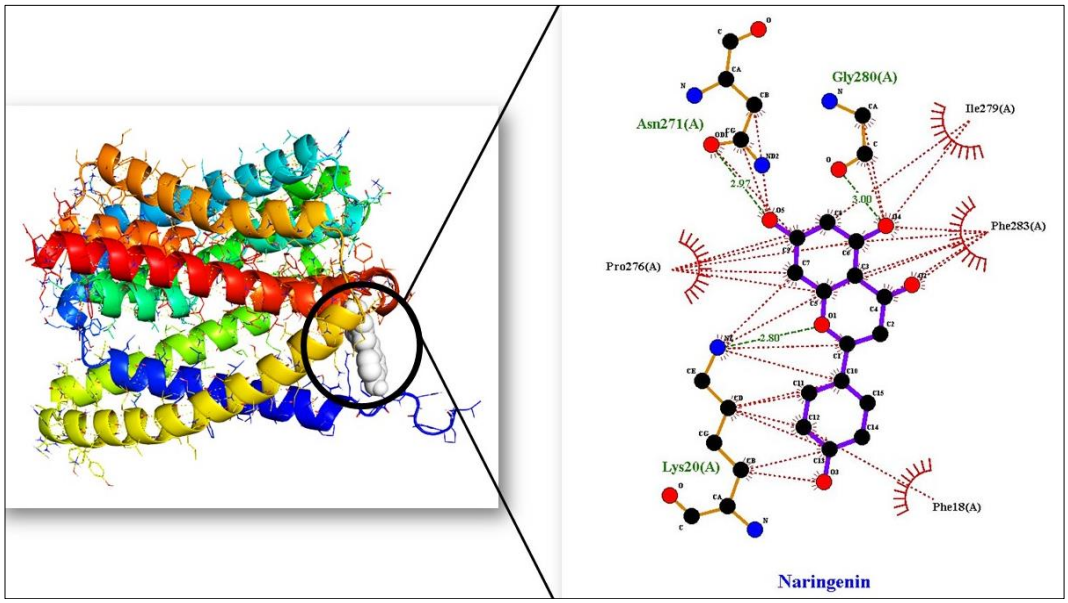
(b)


Gly102(A)
Asn103(A)
Asn262(A)
Leu35(A)
Ser162(A)
Leu31(A)
Val32(A)
Ser28(A)
Ellagic acid

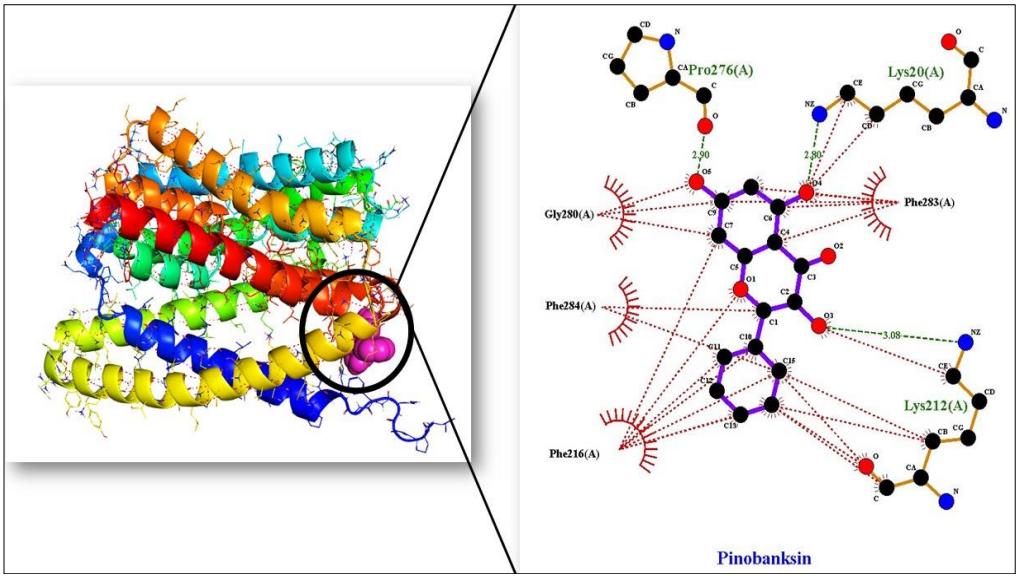
(c)


Phe234(A)
Leu235(A)
Ser255(A)
Met112(A)
Tyr238(A)
Arg252(A)
Phe304(A)
Ferulic acid

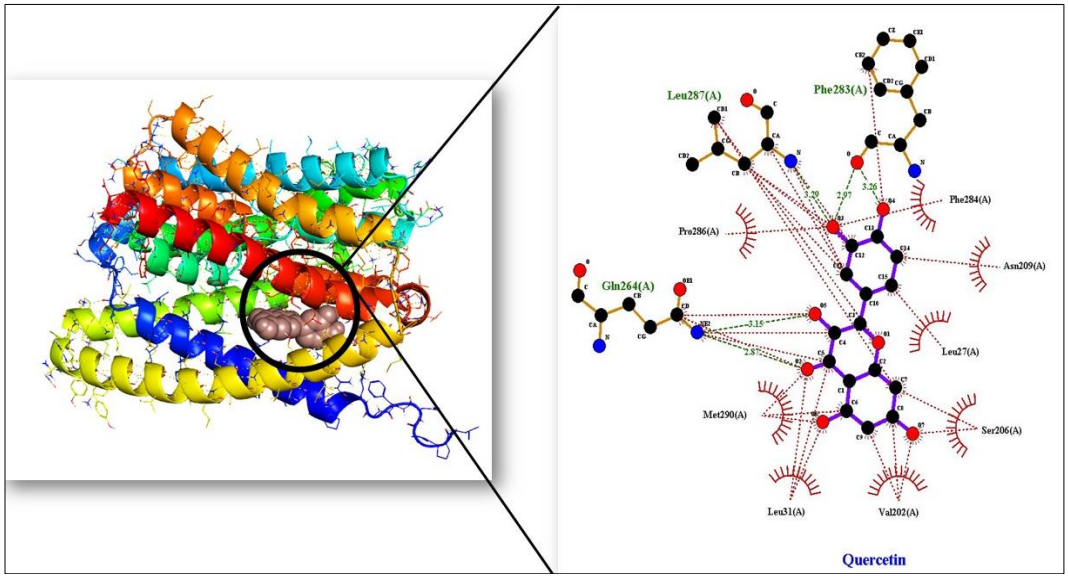
(d)


Gly280(A)
Ile279(A)
Asn271(A)
Phe283(A)
Pro276(A)
Lys20(A)
Phe18(A)
Naringenin

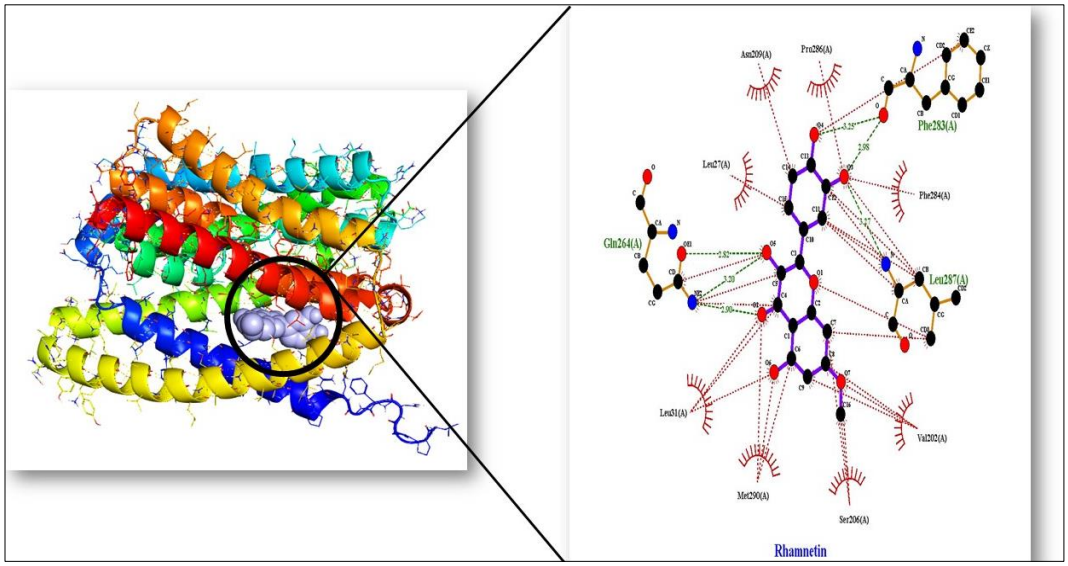
(e)


Pro276(A)
Lys20(A)
Gly280(A)
Phe283(A)
Phe284(A)
Lys212(A)
Phe216(A)
Pinobanksin

(f)



(g)



(h)

Fig 5: Two-dimensional representation of ligand-protein interaction. The red dotted line depicts hydrophobic interaction of ligand with respective amino acid of protein while the green line shows the H_2 bonding of the phytochemical ligand with the amino acid of the target protein, NTCP. **(**a) Caffeic acid interaction (b) ellagic acid interaction (c) Ferulic acid interaction (d) Naringenin interaction (e) p-coumaric acid interaction (f) Pinobanksin interaction (g) Quercetin interaction (h) Rhamnetin interaction

Table 6: MW: Molecular Weight; LogS: Predicted aqueous solubility; Log0/w: Estimated Lipophilicity; Accept H: Solute's predicted affinity for forming hydrogen bonds with water molecules in aqueous solution; Donor H: The number of hydrogen bonds that the solute is expected to provide to water molecules in an aqueous solution; TPSA: Topological polar surface area, molecular descriptor for drug transport properties such as GI absorption.

Ligand Name	MW	Pubchem ID	LogS	Logo/w	Accept H	Donor H	TPSA (Å)	GI absorption
Ferulic acid	194.18	445858	-2.11	1.51	4	2	66.76	High
Quercetin	302.23	5280343	-3.16	1.54	7	5	131.16	High
Rhamnetin	316.26	5281691	-3.36	1.87	7	4	120.36	High
Ellagic acid	302.19	5281855	-2.94	1.10	8	4	141.34	High
p-coumaric acid	164.16	637542	-2.02	1.46	3	2	57.53	High
Caffeic Acid	180.16	689043	-1.89	1.15	4	3	77.76	High
Pinobanksin	272.25	73202	-2.95	1.66	5	3	86.99	High
Naringenin	272.25	932	-3.49	2.52	5	3	86.99	High

3.4 ADME/T analysis of compounds

Pharmacological and pharmacokinetic profiling is considered as an important step in drug development as it helps to predict the efficacy of drug such as its absorbability, bioavailability, its ability to reach site of action, metabolism and finally its excretion that too without posing significant side effect. Several factors are taken into consideration to determine a compound's drug likeness. Computational programs are used widely in the field of pharmaceuticals to test the ADME/T of a compound which aid in selection of top candidates. The

striking aspect of the selected drug compound is their BBB permeability, molecular weight, topological polar surface area (TPSA), LogS value follows the Lipinski rule of five[21] (Table 6). In addition, polar surface area, oral bioavailability, and hydrogen bond donors and acceptors are essential formulation features for medicinal agents. The indicated parameters reveal the qualitative evaluation and rating of absorption, the effects of formulation on drug permeability, the determination of permeability mechanism(s), and the likelihood of transporter-mediated drug-drug interactions. By Lipinski's rule of five, ligands with a strong docking score and gastrointestinal absorption have drug-like characteristics. The BOILED-Egg used for prediction of gastrointestinal absorption and blood brain barrier permeability is given in figure 6. Various parameters of pharmacokinetics are depicted in figure 7.

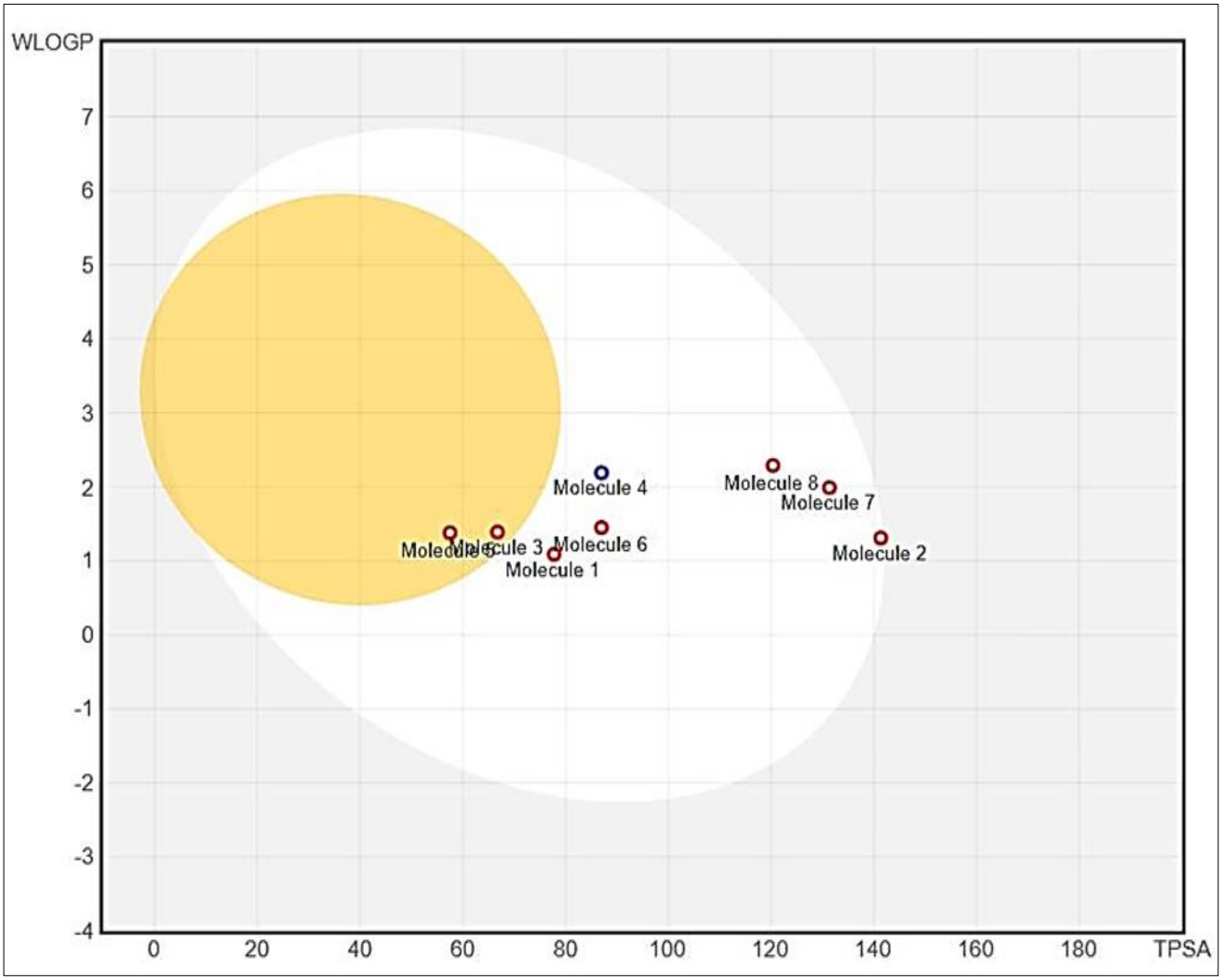


Fig 6: BOILED-Egg to assess GI absorption and blood-brain barrier permeability Yolk: Blood brain permeability; White: GI absorbability; Molecule1: Caffeic acid; Molecule2: Ellagic acid; Molecule3: Ferulic acid; Molecule4: Naringenin; Molecule5: p-coumaric acid; Molecule6: Pinobanksin; Molecule7: Quercetin; Molecule8: Rhamnetin

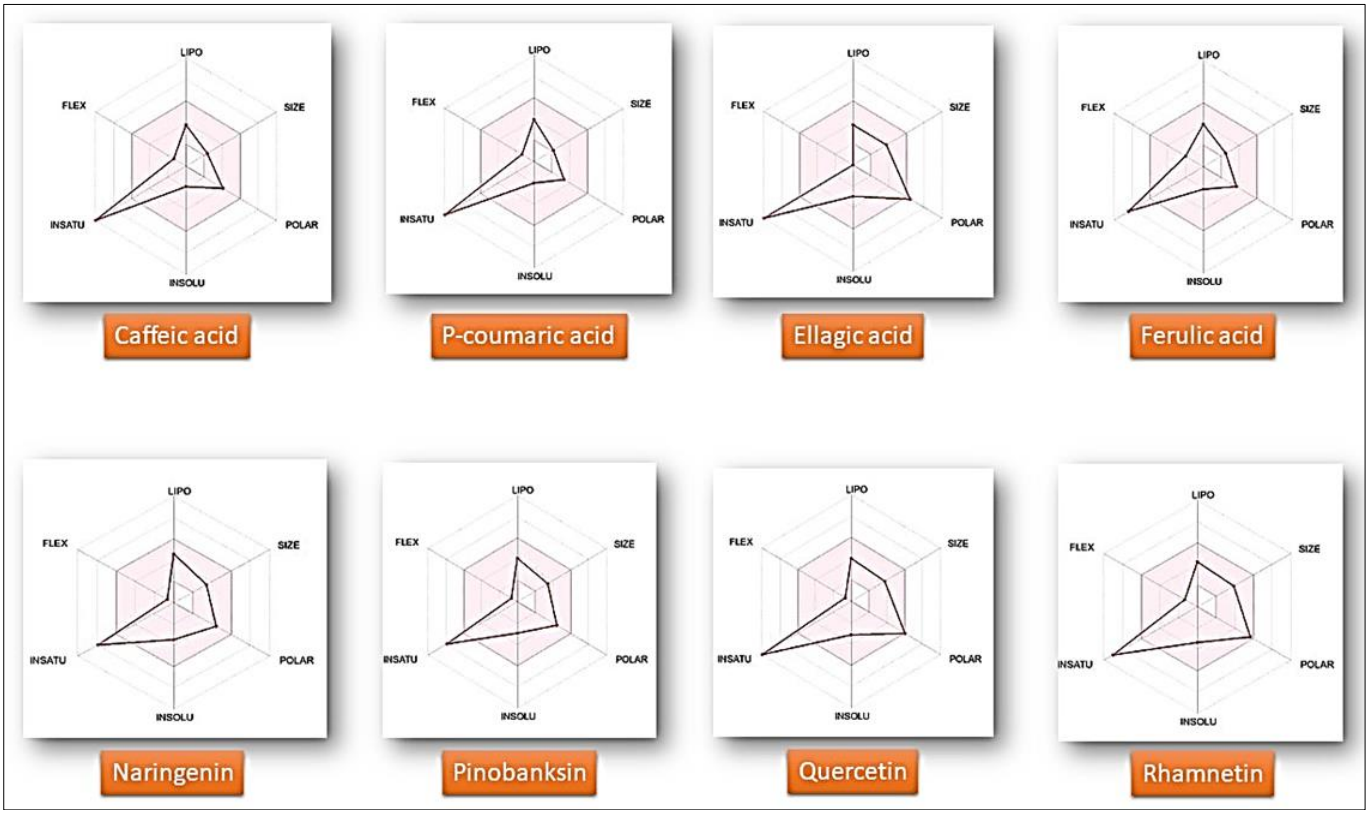


Fig 7: The compound chosen are in the coloured zone which predict their suitable physiochemical space for oral bioavailability and exhibit the INSATU (Instauration), LIPO (Lipophilicity), POLAR (Polarity), SIZE (Molecular Weight), INSOLU (Insolubility), and FLIX (Rotatable bond flexibility) parameters.

4. Discussion

An estimated 296 million individuals worldwide have a chronic infection with hepatitis B virus, and many develop severe liver conditions including cirrhosis, hepatitis, and hepatocellular cancer. NTCP act as gateway for the hepatitis virus and may be studied as a target for therapeutic interventions. Several phytochemicals are in queue to be considered as potent drug candidates. Leaf extract of *Trapa natans* has shown to be having properties such as hepatoprotective and anti-inflammatory. In silico analysis of phytochemicals present in plant leaf extract seems to show striking docking score and ADME/T properties. The data from the study could be used for further validation and formulation.

5. Conflict of Interest: The authors declare no conflict of interest

6. Reference

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