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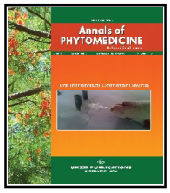

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# Potential inhibitor of *Camellia sinensis* (L.) O. Kuntze phytochemicals on hepatitis C virus NS5B: *In silico* approach

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**Abstract**

Chronically, the hepatitis C virus (HCV) infected people worldwide by around 180 million. HCV is a chronic condition leading to causes liver diseases and if this case final destination is liver transplantation world-wide. We do not have a protective vaccine for HCV treatment till now, but several recent drugs are available, such as boceprevir, ribavirin, sofosbuvir, telaprevir and pegylated interferon (PEG-IFN). Current treatment for HCV is challenging to reach infected individuals because the drug cost is high and has more side effects. This study focused on the phytoconstituents of *Camellia sinensis* (L.) O. Kuntze synonym green tea, which comes under the Theaceae family. This study aims to screen the phytoconstituents for the novel anti-HCV based on the anticipated ligand's favoured orientation against the receptor to make a stable complex-molecular docking analysis using Maestro 12.7 software (Schrodinger, LLC, NY, USA, 2009). We used the Qikprop 6.7 tool to determine the ligand's drug-likeness properties. We screened fifty-one phytochemicals from the *C.sinensis* for their pharmacokinetic properties and evaluated the anti-HCV activity based on the binding energy compared to the reference drug sofosbuvir (–7.541 kcal/mol). The result shows that β-glucogallin (–8.174 kcal/mol), myricetin (–7.987 kcal/mol), and (+)-gallocatechin (– 7.777 kcal/mol). These phytoconstituents lead to significant activity based on the lowest binding energy and inhibit the enzyme activity. So, this work is producing a promising drug candidate for antiviral activity against HCV NS5B polymerase enzyme.

## 1. Introduction

Chronically, the hepatitis C virus (HCV) infected people worldwide by around 180 million. HCV is a chronic condition leading to causes liver diseases and if this case final destination is liver transplantations worldwide (Shepard *et al.*, 2005). We do not have a vaccine to protect the HCV until now, but several recent drugs are available, such as boceprevir, ribavirin, sofosbuvir, telaprevir and pegylated interferon (PEG IFN) (Salama *et al.*, 2016). Sofosbuvir can inhibit nucleotide, competitively block the NS5B polymerase enzyme and inhibits HCV-RNA synthesis by chain termination of RNA. Current treatment for HCV is challenging to reach infected individuals due to the high cost and more side effects. To overcome this problem, we need to discover new substances from the plant source that are less expensive, non-toxic and highly effective in combating HCV. Since ancient times, many countries, Greece, Egypt, India and China, have used traditional medicines from plant products. Medicinal plants source has been considered alternative drugs for treating various human diseases, including liver diseases (Palumbo, 2011). In our study, we have chosen the plant, *C. sinensis* or green tea comes under the Theaceae family. It is a used liquid refreshment in East Asia. People were employed for herbal home remedies used in Europe and North America. It has a great medicinal value as an anti-inflammatory, antioxidant and anticancer (Benelli *et al.*, 2002; Weisburger *et al.*,2002) and is also reported anti-HIV (Hashimoto *et al.*,1996), anti-topoisomerase (Suzuki *et al.*, 2001) antiobesity (Murase *et al.*, 2002) and hypocholesterolemic activities (Ikeda *et al.*, 2003). All tea types are prepared using various oxidation processes: green tea-unfermented, oolong tea-semifermented and black tea - well fermented are collected from *C. sinensis* (Namita *et al.*, 2012; Nakai *et al.*, 2005). Green tea contains more catechins (polyphenols and flavanols), but it decomposes during the production of black tea due to oxidation. The high complexation harmony formed with some metal ions and biological molecules by polyphenols reactive with oxygen (Yang *et al.*,1993). Molecular docking is a method that anticipates ligand's favoured orientation against receptor to make a stable complex (Lengauer *et al.*, 1996). Docking is often applied to predict drug candidates' critical orientation against protein targets and the drug's related activity. Therefore, the molecular docking study is prime for drug design and discovery (Kitchen *et al.*, 2004). The present work investigates the potential antiviral properties of *C. sinensis* phytocompounds. The *in silico* approach examines the mode of interaction between each phytocompound and the target protein NS5B polymerase. That discovered antiviral agents used for dreadful disease inhibitors in future.

## 2. Materials and Methods

### 2.1 Protein preparation for docking study

We used the protein preparation wizard in maestro software (Schrödinger 2021-1) to prepare the protein structure. The crystal

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structure of the NS5B polymerase complex (PDB ID: 3CJ5, 1.92 Å resolution) was down loaded (https://www.rcsb.org/structure/3cj5). The protein preparation preprocesses assigning the hydrogen bond orders, hydrogen atoms addition and deleting the water molecules beyond 5 Å from the hetro group. It optimized the OPLS-3e force field utilized for heavy and hydrogen atoms.The depreciation is restrained to the input protein coordinates by a pre-defined Root mean square deviation (RMSD) tolerance of 0.3 Å(Kalirajan *et al*., 2017; Choudhary *et al.*, 2020; Madhavi Sastry *et al.*, 2013).

### 2.2 Receptor grid generation

The prepared protein structure was used for receptor grid generation at the centroid of the co-crystallized ligand at a maximum length of 20 Å cutoff. Here, we utilized an advanced setting for docking ligands with the dimensions (x-axis 10 Å; y-axis 10 Å; z axis 9 Å) to make a binding box. We have to run the protocol of the rigid receptor docking in extra precision (XP) mode based on the Glide utilized OPLS-3e force field.While the docking operation, the protein was fixed, but ligands were flexible (Sahayarayan *et al.*, 2021).

### 2.3 Ligand preparation

We retrieved 2D structures of fifty-one phytoconstituents of *C. sinensis* from the Pub Chem database in "SDF" (structures data file) format. The ligprepmodule was used for ligand preparation in Maestro 12.7. The force field (OPLS_3e) geometry is optimized in the protein molecule and ideally generates partial atomic charges at the tautomers and ionization states (Sahayarayan *et al.*, 2021).

### 2.4 ADME/Tparameters prediction

The drug-likeness properties of our sort-out compounds are determined by the QikProp 6.7 module of Maestro (Choudhary *et al.*,2020). It evaluates the eligibility of the compounds based on "Lipinski's rule" or "RO5"and "Rule of Three"used to check the drug-likeness properties. We also considered #rotor, CNS, QPlogHERG, brain and blood partition co-efficient (QlogBB), QPlogKp, per cent human oral absorption, human oral absorption, and polar surface area (PSA) to evaluate the potentiality and drug-likeness of ligands (Venkatesan *et al.*, 2018).

### 2.5 Molecular docking

The Glide 9.0 version was used for docking with previously prepared grid-generated protein. The binding energy and affinity evaluated between the ligand and the receptor were scored using the Glide. We set the extra precision (XP) mode and OPLS-3e force field for the docking calculations. The docking process was in a flexible docking mode, developing conformations that automatically enter each ligand input. The generated ligand poses to go through the hierarchal filters that assess the ligand's interaction with the receptor. The beginning filter is the perfect ligand spatial fit to the defined active site. This algorithm acknowledges the favourable hydrophobic site, hydrogen bonding and interactions of the metal-ligation. The more negative value indicates the better binding energy, the XP-Glide score of the phytoconstituents and the fitness scores of each ligand are compared with standard sofosbuvir (Kalirajan *et al.*, 2017). Binding energy is represented in kcal/mol.

### 2.6 Analysis of molecular mechanics/generalized born surface area (MM/GBSA) free energy

The relative binding-free energy (ΔG bind) calculated in each pose docked ligands were rescored by the prime MM/GBSA Version 4.8 tool. In this study, we predict the binding free energy of all hits phytoconstituents on the NS5B polymerase enzyme. The inputs are docked poses for the energy minimization of the free protein, and the free ligands and protein lig and complexes were taken. It also detects the ligand strain energy by setting down the ligand in a solution which was self-generated by VSGB 2.0 suit (Choudhary *et al.*, 2020).

## 3. Results

The various computational software tools forecast the binding relationship between the ligands and the target protein by *in silico* approach (Kumar and Doss, 2016). The traditional medicinal plant *C. sinensis* has also been used to treat various diseases for several years. Mathew *et al.* (2014) and Sangeetha and Rajarajan (2015) have reported that phytochemicals proved antiviral activity against many viruses. Here, we selected a total of fifty-one phytochemicals from the literature. We retrieved all the phytochemicals structures in "SDF" format from the Pub Chem database. Phytochemicals were listed in (Table 1), including molecular formula, structures (Supplementary data Figure S1) and Pub Chem ID.

**Table 1: List of phytochemicals from the *C. sinensis***

| S.No | Phytoconstituents name | Pub Chem ID | Molecular formula |
|---|---|---|---|
| 1 | Catechin | 9064 | $C_{15}H_{14}O_6$ |
| 2 | Epigallocatechin-3-gallate | 65064 | $C_{22}H_{18}O_{11}$ |
| 3 | Epigallocatechin | 72277 | $C_{15}H_{14}O_7$ |
| 4 | Epicatechin gallate | 107905 | $C_{22}H_{18}O_{10}$ |
| 5 | Caffeine | 2519 | $C_8H_{10}N_4O_2$ |
| 6 | Rutin | 5280805 | $C_{27}H_{30}O_{16}$ |
| 7 | Apigenin glycoside | 44257854 | $C_{30}H_{26}O_{12}$ |
| 8 | Flavonol 3-O-d-glucoside (FOG) | 11953828 | $C_{21}H_{20}O_8$ |
| 9 | Myricetin 3-glucoside (M-G) | 44259426 | $C_{21}H_{20}O_{13}$ |
| 10 | Kaempferol | 5280863 | $C_{15}H_{10}O_6$ |

| | | | |
|---|---|---|---|
| 11 | Myricetin | 5281672 | $C_{15}H_{10}O_8$ |
| 12 | Kaempferol-3-O-glucoside | 5282102 | $C_{21}H_{20}O_{11}$ |
| 13 | Quercetin | 5280343 | $C_{15}H_{10}O_{71}$ |
| 14 | (-)-Epigallocatechin3-(3-methyl-gallate)(3Me-EGCG) | 9804842 | $C_{23}H_{20}O_{11}$ |
| 15 | Chlorogenic acid | 1794427 | $C_{16}H_{18}O_9$ |
| 16 | Coumaric acid | 1549106 | $C_9H_8O_3$ |
| 17 | Caffeic acid | 689043 | $C_9H_8O_4$ |
| 18 | Gallic acid | 370 | $C_7H_6O_5$ |
| 19 | Isoschaftoside | 3084995 | $C_{26}H_{28}O_{14}$ |
| 20 | Strictinin | 73330 | $C_{27}H_{22}O_{18}$ |
| 21 | 1,4,6-tri-O-galloyl-β-D-glucose | 10077822 | $C_{27}H_{24}O_{18}$ |
| 22 | β-glucogallin | 124021 | $C_{13}H_{16}O_{10}$ |
| 23 | Theogallin | 442988 | $C_{14}H_{16}O_{10}$ |
| 24 | Theaflavin 3,32 -di-o-gallate | 136055243 | $C_{43}H_{32}O_{20}$ |
| 25 | Theaflavin 32 -O-gallate | 71307578 | $C_{36}H_{32}O_{15}$ |
| 26 | Theaflavin | 135403798 | $C_{29}H_{24}O_{12}$ |
| 27 | Dehydrodicatechin A | 182270 | $C_{30}H_{24}O_{12}$ |
| 28 | Oolongtheanin | 100936097 | $C_{43}H_{32}O_{21}$ |
| 29 | Theasinensin E | 467317 | $C_{30}H_{26}O_{14}$ |
| 30 | Theasinensin D | 442543 | $C_{44}H_{34}O_{22}$ |
| 31 | Theasinensin B | 467315 | $C_{37}H_{30}O_{18}$ |
| 32 | Oolonghomobisflavan B | 14520995 | $C_{45}H_{36}O_{22}$ |
| 33 | Oolonghomobisflavan A | 14520989 | $C_{45}H_{36}O_{22}$ |
| 34 | Assamicain B | 467310 | $C_{44}H_{36}O_{22}$ |
| 35 | Procyanidin B-2 | 5320711 | $C_{30}H_{26}O_{12}$ |
| 36 | Procyanidin B-3 | 146798 | $C_{30}H_{26}O_{12}$ |
| 37 | Prodelphinidin B-2 | 467304 | $C_{30}H_{26}O_{14}$ |
| 38 | Prodelphinidin B-2 3,32 -di-O-gallate or rhodisin | 467306 | $C_{44}H_{34}O_{22}$ |
| 39 | Prodelphinidin B-4 | 467307 | $C_{37}H_{30}O_{18}$ |
| 40 | (e)-Epigallocatechin (4β-8)-(e)-epicatechin 3-O-gallate | 442678 | $C_{37}H_{30}O_{17}$ |
| 41 | (−)-Epiafzelechin3-O-gallate | 467295 | $C_{22}H_{18}O_9$ |
| 42 | (−)-Epicatechin | 14015928 | $C_{45}H_{36}O_{18}$ |
| 43 | (−)-Epicatechin 3-O-gallate | 131752218 | $C_{66}H_{50}O_{30}$ |
| 44 | (−)-Epigallocatechin 3-O-gallate | 101834717 | $C_{44}H_{34}O_{21}$ |
| 45 | (−)-Epigallocatechin 3,5-di-O-gallate | 467299 | $C_{29}H_{22}O_{15}$ |
| 46 | (−)-Epigallocatechin 3-O-p-coumaroate | 6474788 | $C_{24}H_{20}O_9$ |
| 47 | (−)-Catechin 3-O-gallate | 44257105 | $C_{22}H_{18}O_{10}$ |
| 48 | (+)-Gallocatechin | 65084 | $C_{15}H_{14}O_7$ |
| 49 | (−)-Gallocatechin 3-O-gallate | 44257114 | $C_{22}H_{18}O_{11}$ |
| 50 | C-Ascorbyl(−)-epigallocatechin | 3001587 | $C_{21}H_{20}O_{13}$ |
| 51 | C-Ascorbyl(−)-epigallocatechin 3-O-gallate | 14520973 | $C_{28}H_{24}O_{17}$ |

### 3.1 ADME/T study for *C.sinensis* phytochemicals

As per Lipinski's rule, the unfit phytoconstituents were rejected, determining the similar known drugs (Lipinski *et al*., 2012; Mishra *et al*., 2018). The "Rule of 5" interfered with physicochemical parameters were essential and determined (Arumugam *et al*., 2012). This work listed the molecular weight within the limit ligands (M.W. d" 500). The principle of low molecular weight molecules can be easily achieved pharmacokinetic properties compared to heavy molecular weight molecules. The drug's potency indicates that the Log P (lipophilicity) value, H-bond donors and H-bond acceptors are shown within the limit. The molecular flexibility parameter indicates rotatable bonds (# rotor). Thus, the number of rotatable bonds (NRB) was used as an additional criterion for favourable drug metabolism and pharmacokinetics properties (Ntie-Kang *et al.*, 2013). Here, The # rotor values are in an acceptable range of 0-10. The $CNS^+$ compounds inferred the blood-brain barrier to induce CNS hurt side effects. In contrast, the $CNS^-$ compounds are tough to reach (Luco and Juan, 1999). This study reported phytochemicals as – 2, indicating CNS inactive except for caffeine. Jorgensen's 'Rule of 3' is more likely to be orally bioavailability. The listed compound complies with "RO3" (Jorgensen and Duffy, 2000; Jorgensen and Duffy, 2002; Schrödinger, 2011d). *C. sinensis* phytochemicals reveal log $S_{wat}$ ranges from – 0.714 to – 3.841. The caffeine had great QPPcaco-2 permeability in the gut-blood barrier and remaining phytochemicals in the acceptable content. Primary metabolites of epicatechin gallate had 9, and other compounds are in a reasonable range. The quantitative prediction of the human oral absorption parameter reveals most of the compound medium in coverage. The predicted per cent of human oral absorption (on a 0 to 100% scale) (Ntie-Kang *et al.*, 2013). Distribution of log B/B brain/blood displayed compounds fall within the recommended range. The skin permeability parameter distribution represents log Kp (– 8.0 to – 1.0). Here, phytochemicals are less than – 7.299. The log KHSA (blood plasma proteins) represent drugs binding to plasma proteins, considerably reducing the quantity of the drug entering the systemic circulation. In our study, the values showed that plasma protein binding phytochemicals had within the limit. The potassium ion ( K+) channel encodes on the human ether-a-go-go-related gene (HERG). In the fatal arrhythmia called 'Torsade de Pointes' or 'Long QT' syndrome (Hedley *et al.*, 2009). Vandenberg *et al.* (2001) and Chiesa *et al*. (1997) have reported cardiac and leukemic cell toxicity. So, the range for predicted log $IC_{50}$ values blockage of HERG K+ channels (log HERG) is below – 5. In this parameter, the phytochemicals are in an acceptable range. We determined the polar surface area of nitrogen, oxygen and carbonyl carbon atoms and listed phytochemicals within a limit. "Rule of five" and "Rule of Three" maximum 4 and 3, respectively. Only fifteen phytochemicals passed the acceptable range out of fifty-one phytochemicals.

### 3.2 Molecular docking study for *C. sinensis* and NS5B polymerase protein

The molecular docking analysis of *C. sinensis* phytochemicals (total of 51) docked against NS5B polymerase HCV protein. Around 11 compounds have shown a Glide score above – 5 and good NS5B inhibition activity. Table 2 displayed the Docking score, Glide ligand efficiency, Interaction residues, $Glide_{evdw}$, Glide energy, H-bond distance (Å), Pi-Pi stacking, and Pi cation. We selected a type A polypeptide chain for this docking study that targets all the ligands. Ligand efficiency is an extensive criterion for drug detection. It is premeditated by scaling affinity by molecular size (Kenny, 2019). Glide ligand efficiency of phytochemicals was determined to range from – 0.189 to 0.485. *C.sinensis* phytochemicals of $Glide_{evdw}$ were detected at values – 19.42 to – 30.812 and Glide energy from – 25.485 to – 43.191.

Here, the top 5 complex docking scores are briefly discussed and displayedin 2D and 3D structures. Docking complex between the β-glucogallin and the NS5B polymerase protein (Figure 1). β-glucogallin formed four H-bond with the residues of NS5B, including ARG 501, ARG 498, LEU 474, and TYR477 with a distance of 2.79, 1.89, 1.96 and 1.98Å, respectively, and the score was – 8.174 kcal/mol. Figure 2 represents the binding linkage between the myricetin and protein crucial score of – 7.987 kcal/mol. It produces a hydrogen bond with the residue of ARG 498 and the distance of the H-bond had 2.15Å. It formed Pi-Pi stacking and Pi-cation with TYR 477 and ARG 501. The docked (+)-gallocatechin with NS5B protein complex (Figure 3) forms two hydrogen bonds with the residues of LEU 474 (2.09Å) and ARG 498 (2.12Å) with H-bond distances. It has a binding score of – 7.777 kcal/mol and Pi-Pi stacking formed with TYR 477. Figure 4 shows hydrogen bonding interactions between the catechin and the target protein with two amino acids, LEU 474 and ARG 498, at NS5B active site, H- bond distances are 2.10 and 1.98 Å. It makes Pi-Pi stacking between ligand and TYR 477 and docking score – 7.267 kcal/mol. Figure 5 illustrates the complex formed between the flavonol 3-O-d-glucoside and the target protein with the docking score of – 7.048 kcal/mol *via* four H-bond linkages with the residues: TRP 528, LYS 533, SER 476 and TYR 477, with H-bond distances of 1.97, 1.82, 2.54 and 1.88 Å, respectively.

Table 2 shows kaempferol to chlorogenic acid docking figures in supplementary S2. Kaempferol formed two H-bonds with the residues of NS5B, including ARG 498 and LEU 474, with a distance of 2.05 Å and 2.13 Å. The docking score was – 6.9 kcal/mol, forming Pi-Pi stacking with TYR 477 and ARG 501 makes two Pi cations. The binding relationship between the epigallocatechin and protein complex energy was – 6.799 kcal/mol. The hydrogen bond was found at ARG 498 with a distance of 1.94 Å. The docked complex of kaempferol-3-O-glucoside on target protein binds *via* three residues at TYR 477, LYS 533 and LYS 533 (H-bond distances like 2.00, 2.29 and 2.12 Å) and the binding energy of – 6.262 kcal/mol. The complex between the epicatechin gallate and the protein docks with SER 476, TYR 477, LEU 474 and ARG 498 amino acids and the docking score was – 6.059 kcal/mol. Pi cation formed with ARG 501. Gallic acid assembled two H-bonds at the active site of protein with the residues such as LEU 474 and ARG 501, with a distance of 2.08 and 2.68 Å and the docking score was – 5.815 kcal/mol. The

chlorogenic acid docking score was – 5.671 kcal/mol. It formed two hydrogen bonds with ARG 501 and SER 476 amino acids with a distance of 2.34 and 2.07 Å. All the phytochemicals intermediate water molecule bridge involved with ARG 498 at the NS5B polymerase active site, except catechin, gallic acid and chlorogenic acid. In TYR 477 H-bond formed to ligands with water bridge, such as β-glucogallin, flavonol 3-O-d-glucoside,epicatechin gallate and kaempferol-3-O-glucoside. The docking score of references of osbuvir was –7.541 kcal/mol. H-bonds created residues at ARG 501, SER 476, ARG 498 and TYR 477 (Figure 6). This study compares all the ligand's binding energies to the reference sofosbuvir. According to the docking score, we decided on the hit compoundthat β-glucogallin has the best docking score compared to others. Therefore, β-glucogallin(– 8.174kcal/mol) was the most active compound compared to sofosbuvir (– 7.541 kcal/mol) and three hydrogen binding interactions with the ARG 501, ARG 498 and TYR 477 residues of the NS5B active site.

### 3.3 Free energy calculation by prime MM/GBSA

The prime MM/GBSA approach is used for free energy calculation and to better rank the ligands. The values of approximate free energy of binding were obtained after analysis. Table 3 displays the binding energy of the docked ligands at the binding site. Epicatechin gallate expressed more binding energy of – 56.05 kcal/mol compared to the best-docked compound β-glucogallin – 43.06 kcal/mol within the NS5B polymerase active site. We also determine the dG-bind in Coulomb, dG-bind (N.S.) and dG bind (N.S.)-Coulomb. The energy unit is kcal/mol.

**Table 2: Docking results of *C. sinensis* on NS5B polymerase of HCV protein**

| S.No | Compound name | Docking score kcal/mol | Glidelig and efficiency | $Glide_{evdw}$ | Glide energy kcal/mol | Interaction residues | H-bond distance Å | Pi-Pi stacking | Pi cation |
|---|---|---|---|---|---|---|---|---|---|
| 1 | β-glucogallin | – 8.174 | – 0.355 | – 22.516 | – 30.87 | ARG 501<br>ARG 498<br>LEU 474<br>TYR 477 | 2.79<br>1.89<br>1.96<br>1.98 | ---- | ---- |
| 2 | Myricetin | – 7.987 | – 0.347 | – 29.702 | – 38.545 | ARG 498 | 2.15 | TYR 477 | ARG 501 |
| 3 | (+)-gallocatechin | – 7.777 | – 0.353 | – 31.23 | – 40.228 | LEU 474<br>ARG 498 | 2.09<br>2.12 | TYR 477 | ---- |
| 4 | Catechin | – 7.267 | – 0.346 | – 29.824 | – 38.37 | LEU 474<br>ARG 498 | 2.10<br>1.98 | TYR 477 | ---- |
| 5 | Flavonol 3-O-d-glucoside (FOG) | – 7.048 | – 0.243 | – 27.776 | – 40.616 | TRP 528<br>LYS 533<br>SER 476<br>TYR 477 | 1.97<br>1.82<br>2.54<br>1.88 | ---- | ---- |
| 6 | Kaempferol | – 6.9 | – 0.329 | – 28.84 | – 36.611 | ARG 498<br>LEU 474 | 2.05<br>2.13 | TYR 477 | ARG 501<br>ARG 501 |
| 7 | Epigallocatechin | – 6.799 | – 0.309 | – 29.927 | – 39.125 | ARG 498 | 1.94 | ---- | ---- |
| 8 | Kaempferol-3-O-glucoside | – 6.262 | – 0.196 | – 30.812 | – 43.114 | TYR 477<br>LYS 533<br>LYS 533 | 2.00<br>2.29<br>2.12 | ---- | ---- |
| 9 | Epicatechin gallate | – 6.059 | – 0.189 | – 31.911 | – 43.191 | SER 476<br>TYR 477<br>LEU 474<br>ARG 498 | 1.87<br>2.41<br>2.39<br>1.90 | ---- | ARG 501 |
| 10 | Gallic Acid | – 5.815 | – 0.485 | – 19.42 | – 25.485 | LEU 474<br>ARG 501 | 2.08<br>2.68 | ---- | ---- |
| 11 | Chlorogenic acid | – 5.671 | – 0.227 | – 21.939 | – 33.58 | ARG 501<br>SER 476 | 2.34<br>2.07 | ---- | ---- |
| 12 | Sofosbuvir | – 7.541 | – 0.209 | – 31.764 | – 45.485 | ARG501<br>SER 476<br>ARG 498<br>TYR 477 | 2.53<br>2.69<br>2.00<br>2.02 | ---- | ---- |

**Table 3: The binding-free energy calculation by prime MM-GBSA approach**

| Compound | MMGBSA-dG-binding energy | MMGBSA-dG-bind in coulomb | MMGBSA-dG-bind (NS) | MMGBSA-dG bind (NS)-coulomb |
|---|---|---|---|---|
| β-glucogallin | – 43.06 | – 39.88 | – 62.03 | – 62.26 |
| Myricetin | – 41.8 | – 34.89 | – 46.21 | – 32.05 |
| (+)-gallocatechin | – 46.58 | – 35.92 | – 51.43 | – 37.17 |
| Catechin | – 43.33 | – 31.11 | – 51.62 | – 34.58 |
| Flavonol 3-O-d-glucoside (FOG) | – 42.09 | – 38.41 | – 58.19 | – 37.42 |
| Kaempferol | – 41.28 | – 16.88 | – 48.35 | – 14.78 |
| Epigallocatechin | – 44.96 | – 36.92 | – 49.22 | – 35.54 |
| Kaempferol-3-O-glucoside | – 41.31 | – 18.93 | – 55.64 | – 28.37 |
| Epicatechin gallate | – 56.05 | – 52.04 | – 62.87 | – 46.51 |
| Gallic acid | – 32.19 | – 17.6 | – 41.03 | – 21.42 |
| Chlorogenic acid | – 35.69 | – 37.39 | – 49.32 | -36.5 |

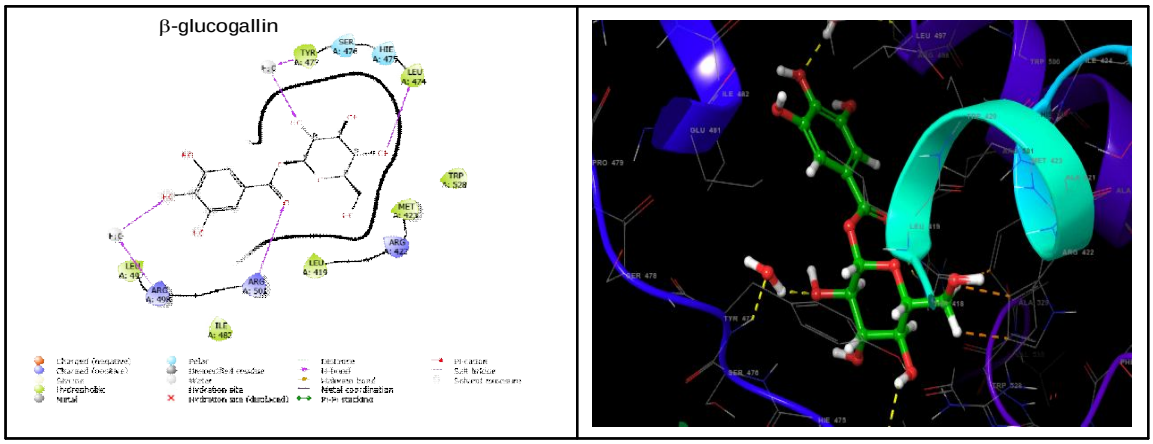


**Figure 1: 2D and 3D structure of molecular docking complex of β-glucogallin against NS5b polymerase protein. In 3D secondary protein molecules-ribbon backbone, ball and stick - ligands. In 2D format, the purple line indicates H-bond; The 3D diagram with yellow dotted lines-hydrogen bond. In the ligand, white-hydrogen, green-carbon and red-oxygen.**

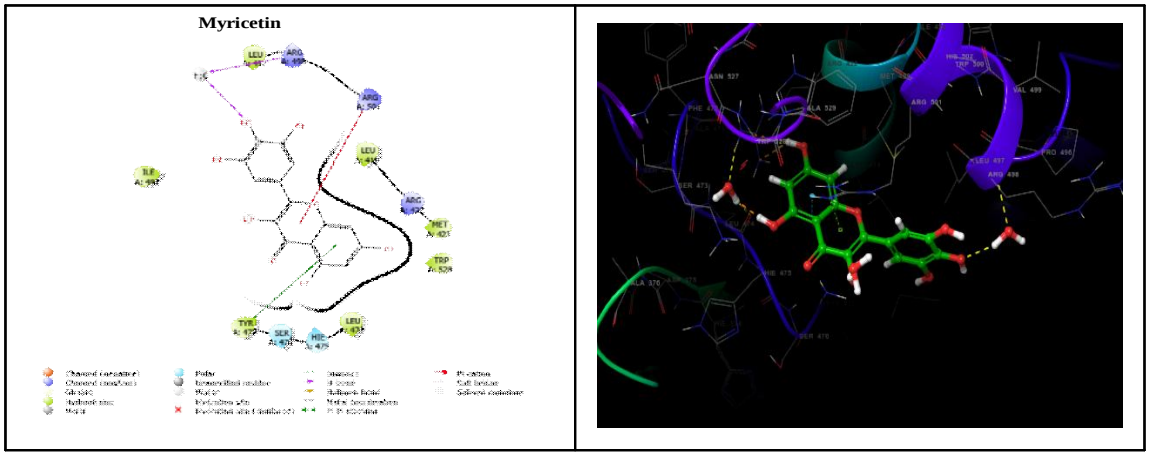


**Figure 2: 2D and 3D structures of myricetin against NS5b polymerase complex. In 2D format, the purple line-H-bond; The green line-Pi-Pi stacking; The red line-Pi cation. The 3D diagram is given with yellow dotted lines representing hydrogen bond; Sky blue dottedlines-pi-pi stacking.**

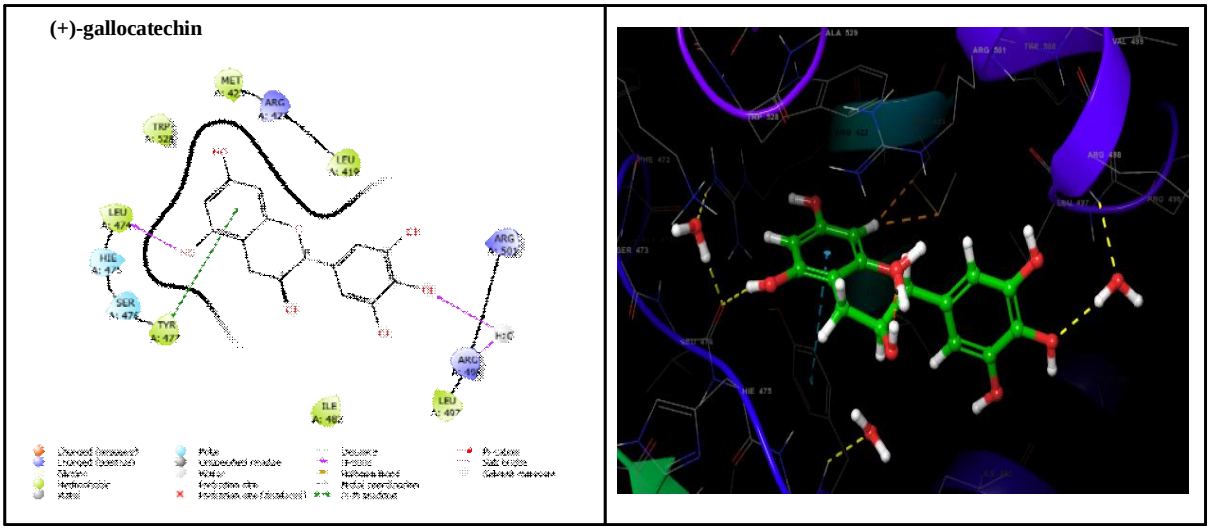


**Figure 3:** Molecular docking studies of 2D and 3D structures of (+)-gallocatechin against NS5b polymerase protein.

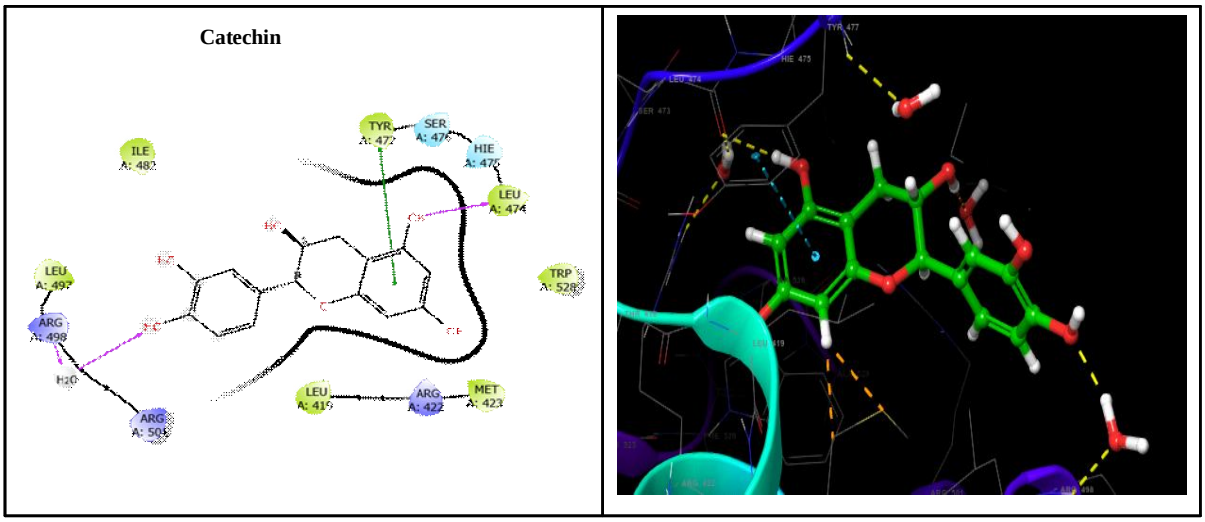


**Figure 4:** 2D and 3D structures of catechin against NS5b polymerase protein complex.

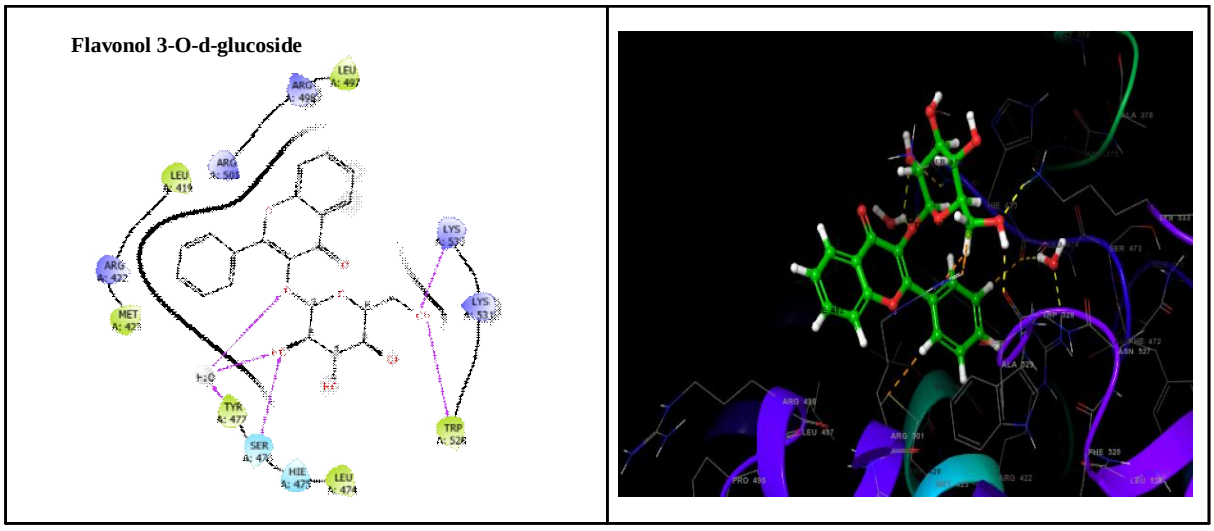


**Figure 5:** Molecular docking studies of 2D and 3D structures of flavonol 3-O-d-glucoside against NS5B polymerase complex.

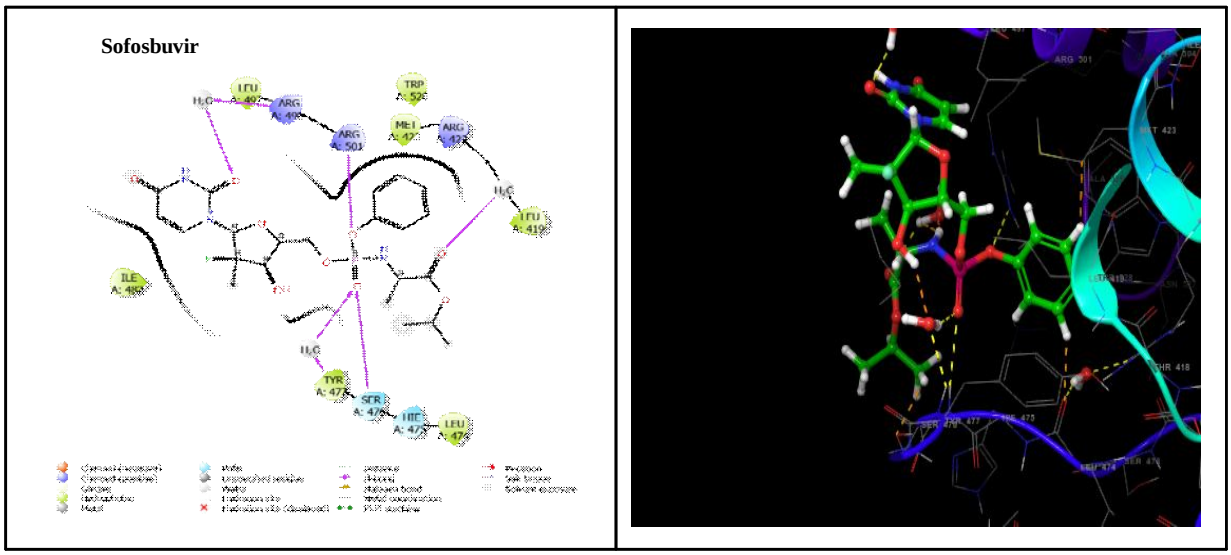


**Figure 6: Molecular docking studies of 2D and 3D structures of SOFOSBUVIR against NS5B polymerase protein complex.**

## 4. Discussion

We determined the lead hit phytoconstituents from *C. sinensis* on the HCV NS5B polymerase target protein. The advantage is that plant bioactive compounds are safer, non toxic and cheaper than synthetic drugs (Kortemme *et al.*, 2003). This study also explained that more excellent hydrogen bond interaction residues between the protein and ligand regulate crucial strength. The active functional group of ligands fit into the mandatory pocket of the protein *via* hydrogen bonding interactions (Jin *et al.*, 2014). The docking analysis of *C.sinensis* reports out phytochemicals of β-glucogallin, myricetin and (+)-gallocatechin has the best docking score with an excellent ADME/T profile. Boyce *et al.*(2014) reported the significance of thumb domain junction with the C-terminal tail (thumb residues 371-561)**.** Our docking results showed that ligands had hydrogen bond interaction residues ARG 498, LEU 474, TYR 477 and ARG 501 at the thumb domain. These docking results reveal the Non nucleosides inhibitors (NNIs) of the 3CJ5 complex with tiny fragments. Here, the reported ligand β-glucogallin interacts with polar residues TYR 477 and ARG 501 at the allosteric location, mainly the thumb II site. Based on the binding energy and affinity, top hit phytoconstituents inhibited the enzyme polymerase activity at the target protein. In this work, phytochemicals could be a promising drug candidate for antiviral activity against HCV NS5B polymerase enzyme without side effects.

## 5. Conclusion

The *in silico* method curtails the time and cost spent synthesizing and testing compounds before entering the clinical trials. Modern drugs have their lineage in traditional medicines. The Indian system of drugs-based herbs has been gaining importance globally in recent years because of their efficacy, safety, easy accessibility and cost-effectiveness. ADMET properties have one of the integrated factors of the drug discovery system, guiding lead selection and optimization. We target the NS5B polymerase because essential for viral replication. The present study screened the phytochemicals from *C. sinensis*. The unfit ligands were rejected based on the ADME/T parameters, and then the molecular docking target is less binding energy and more affinity was achieved. The low binding energy phytoconstituents inhibit enzyme activity, β-glucogallin, myricetin and (+)-gallocatechin. These top three leads show the lowest binding energy and great affinity satisfied by the studied parameters. In addition to the lead-like characteristics, β-glucogallin has demonstrated an excellent docking score with a perfect ADME/T profile. So, we conclude that the top three hit phytoconstituents are promising drug candidates for developing anti-HCV drugs.

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## Conflict of Interest

The authors declare no conflicts of interest relevant to this article.

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