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IN SILICO INVESTIGATION OF MUNG BEAN (*Vigna radiata* L.) ACTIVE COMPOUNDS AS POTENTIAL NATURAL INHIBITOR AGAINST HEPATITIS C VIRUS E2 ENVELOPE GLYCOPROTEIN

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Abstract

The hepatitis C virus (HCV) is the causative agent of hepatitis C, an infection of the liver. The liver may suffer long-term damage as a result of this illness. A significant number of people in Indonesia are infected with hepatitis C. Because of the restrictions and potential side effects of their hepatitis C treatment, a large number of people decide to stop receiving medication. Traditional remedies made from plants have started to become more popular in recent years due to the fact that they are less expensive and have less adverse effects. Thus, in this present study we aimed to evaluate the *Vigna radiata* active constituent as possible anti-viral drug against Hepatitis C Virus Envelope E2 Glycoprotein through *in silico* approaches. The Lipinski rule of five is used in the process of virtually screening compounds to identify those that have properties similar to candidate of drug. Following that, the target chemicals and proteins were adjusted in preparation for additional molecular docking. In order to get binding affinity scores, chemical interactions, ligands sites, and amino acid residues, visualization and data analysis were carried out. Based on our investigation, we found top five molecules with lower binding energy values than IFN-α as control drug including isovitexin, orientin, vitexin, caffeic acid, and p-coumaric acid. In addition, the findings demonstrated that the chemical bonds that facilitate the contact process are present in every substance that forms a binding interaction with the target protein. As a result, our findings provide preliminary evidence that the *V. radiata* plant has the potential to act as an anti-viral medicine that is effective against hepatitis C virus E2 envelope glycoprotein.

INTRODUCTION

Hepatitis C is an infection of the liver brought on by the hepatitis C virus [1]. HCV infects about 150 million people worldwide; up to 350,000 people die each year [2]. Indonesia has a high prevalence of hepatitis C; dominantly people are chronically infected with HCV [3]. Chronic HCV infection can cause fibrosis, cirrhosis, and hepatocellular cancer in the liver [1]. Hepatitis C can be spread by parenteral pathways, blood transfusions, free sex, and intravenous drug

administration [4]. Interferon-alpha (IFN-α), ribavirin (RBV), and direct-acting antiviral therapy (DAA) are currently available therapies [5]. The World Health Organization (WHO) is also trying to tackle hepatitis C by reducing new infections by 90% and deaths by 65%. Some patients infected with HCV are asymptomatic, making efforts to eradicate hepatitis C difficult [6]. Constraints commonly encountered in patients taking hepatitis C treatment are side effects of the drugs given, such as the risk of resistance, limitations in patients with advanced liver disease, transplantation, and immune disorders, causing patients to discontinue therapy for hepatitis C well as unaffordable treatment costs [7].

Hepatitis C Virus Envelope E2 Glycoproteinis an essential mediator in attaching and fusing viruses in host cells [8]. HCV E2 protein is an immunogenic protein that functionally forms heterodimers with HCV E1 protein that facilitates viral fusion into host cells. [9]. Structurally, the HCV E2 protein has two hypervariable regions (HVR), HVR1, which plays a role in target cell recognition and virus attachment to host cells, and HVR2, which helps the binding process of the virus to cell surface receptors [10]. The cell surface receptor interacts directly with the HCV E2 protein is the CD81 receptor [11]. Studies using the HCV E2 and CD81 mutants showed that the infection success rate of these mutants was only 30%, indicating that the CD81 protein is the main protein that facilitates the fusion of HCV into host cells [12]. The interaction of HCV E2 with CD81 protein needs to be inhibited with specific compound inhibitors so that the ratio of infection and reinfection of host cells decreases. There are no antiviral drugs that directly target the HCV E2 protein or its interaction with the CD81 protein.

Plant-based treatment methods containing bioactive compounds have been widely used, mainly because of a holistic approach. Several plants simplicial have been tested for pharmacological properties such as antiviral, antiinflammation, anticancer, fewer side effects, and affordability [13-16]. Bioactive compounds are also found in functional food—foods that can provide health benefits because of their active components [17]. One of them is mung beans (*V. radiata*). Mung beans contain many secondary metabolites, including flavonoids, polyphenols, and peptides [18]. Apart from basic nutritional needs, several studies have looked at the possible advantages of mung beans, such as hypoglycemic and hypolipidemic effects, antihypertensive, anticancer, hepatoprotective, and immunomodulatory effects [19]. This can indicate that mung beans may have potential bioactive compounds as hepatitis C drugs.

Previous research has investigated the potential of plant bioactive compounds against HCV E2 protein as a target for treating hepatitis C [20]. However, no research has examined the potential of mung bean bioactive compounds as a potential hepatitis C drug by targeting the HCV E2 protein. This study used mung bean bioactive compounds to explore these compounds as potential hepatitis C drugs, specifically those targeting the HCV E2 protein in the HCV fusion process.

MATERIALS AND METHODS

Ligands Collection and Drug likeness Verification

The bioactive components of *V. radiata* were particularly gathered through a literature review, including caffeic acid, catechin, chlorogenic acid, cyanidin-3-glucoside, dulcinoside, gallic acid, gentisic acid, isovitexin, kaempferol, luteolin, mycretin, p-Coumaric acid, pelargonidin-3-glucoside, peonidin-3-glucoside, quercetin, sinapic acid, syringic acid, and vitexin. We used IFN-α as an alternative because there is currently no specific drug available that directly targets the E2 protein. All of the substances that were retrieved from PubChem database (https://pubchem.ncbi.nlm.nih.gov/) in SDF format according to our previous study [21].

The Supercomputing Facility for Bioinformatics and Computational Biology at IIT Delhi ran preliminary tests on each compound using Lipinski's Rule of Five parameters (http://scfbio-iitd.res.in/software/drugdesign/lipinski.jsp). These tests were conducted in order to determine whether or not the compound was efficacious. If a substance fulfils all of the criteria outlined in the Lipinski test parameters, including having a molecular mass of less than 500 Daltons, high lipophilicity (with a LogP of no more than 5), having fewer than five hydrogen bond donors, having fewer than ten hydrogen bond acceptors, being refractive, and having a molarity that falls between 40 and 130, then it is considered to have passed the test and continued for the molecular docking. Compounds that are able to pass the Lipinski test are those that possess the necessary chemical and physical pharmacological properties to be administered orally to humans [22].

Target Protein Collection and Docking Process

The HCV E2 protein structure was retrieved from RSCB PDB (https://www.rcsb.org/) in PDB format with ID 6BZY. It is an immunogenic protein that functionally forms heterodimers with HCV E1 protein to mediate cell entry and fusion [9]. We optimized the protein target by removing all of its native ligands by PyMol (https://pymol.org/2/), then saved them in PDB format.

We used AutoDock Vina, which is integrated by PyRx-Virtual Screening Tools 0.8 for the docking process (https://pyrx.sourceforge.io/). Proteins and ligands were minimized and converted into autodock macromolecular format (*pdbqt). We used the blind docking method, which included a whole protein structure with a coverage area about X: 39.7647 Å, Y: 43.7102 Å, and Z: 50.9872 Å, with central coordinate around X: -17.718 Å, Y: -15.997 Å, Z: 13.9888 Å. The docking result is the affinity value measured in kcal/mol.

Data Visualization and Analysis

Visualization of docking results is carried out in two stages: the 3D visualization using PyMol (https://pymol.org/2/) to obtain an overview of each compound's binding site on target protein, and 2D visualization using LigPlot+ v.2.2.4 (https://www.ebi.ac.uk/thornton-srv/software/LigPlus/) to display and determine the type of interaction for each protein-ligand complex.

The analysis of docking results was carried out by comparing the affinity values for potential compounds with control compounds and comparing the types of interactions between potential compounds and control compounds [23,24]. The visualization result is that the binding site residues are compared with the critical binding residues on the HCV E2 protein.

RESULTS AND DISCUSSION

V. radiata or widely known as mung bean is a plant of the Fabaceae that contains minerals, nutrients, protein, vitamins (A, B1, and C), and significant amounts of bioactive compounds [25]. *V. radiata* are used as medicine for hypoglycemic effects, hypolipidemic effects, antihypertensive, anticancer, hepatoprotective, and immunomodulatory [19]. Previous *in vitro* studies have stated that IFN-α can inhibit HCV RNA replication and viral protein translation and inhibit HCV entry by inducing transmembrane protein 1 (IFITM1) [26]. No study stated that IFN-α directly targets the E2 protein to disrupt the virus entry. Therefore, we try to repurpose it to directly inhibit E2 protein in this study. Compared with other hepatitis C therapies that only inhibit the replication process, such as DAA therapy [27].

Five bioactive compounds that have the best affinity against E2 protein were p-coumaric acid (637542), caffeic acid (689043), orientin (5281675), vitexin (5280441), and isovitexin (162350), ranging from -7.6 to -6.0 kcal/mol, which lower than control (IFN-α, -5.9 kcal/mol) (Figure 1). Because the energy released by the potential compounds is higher, they are predicted to have more stable interactions than the control, and the likelihood of interaction between them and E2 protein was higher than the control. The visualization results also revealed that the five possible compounds were all found in the same binding groove, suggesting that their inhibitory potential against the binding area of the HCV E2 protein was identical. The visualization plot showed that all top five compounds and IFN-α interacted in a comparable position on the E2 protein active site, shown in Table 1.

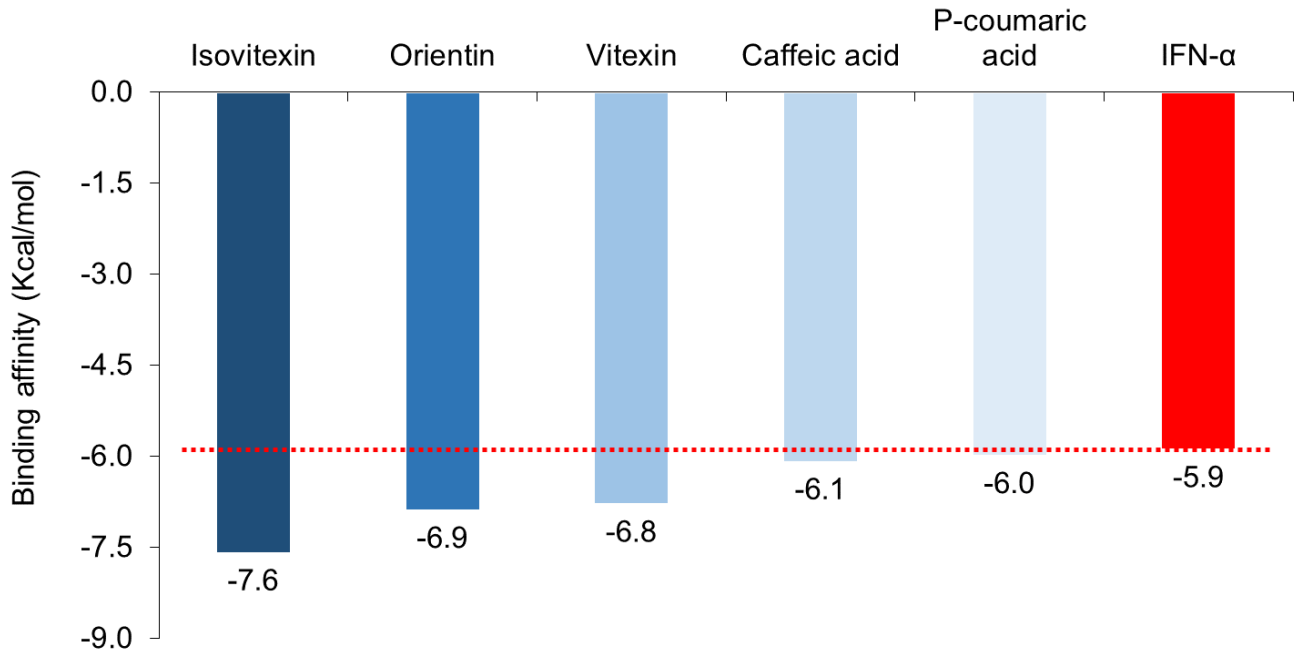

Figure 1. Binding affinity scores among the *V. radiata* compares to control.

Table 1. Docking results of the top five compounds with binding site residue

Bioactive Compound	Binding Residue	Interaction (Å)
Isovitexin **ΔG** = -7.6 kcal/mol	Tyr618, Trp616, Pro619, Met555, Phe447, **Phe437,** Ala440, His445, Gly436, Thr435	Hydrophobic contact
	Thr561	Hydrogen bond (3.00)
Orientin **ΔG** = -6.9 kcal/mol	Thr542, Val514, **Trp420,** Val515, Arg424, Val516, Gly523	Hydrophobic contact
	Asn541 Gly517 Thr519 Ile538	Hydrogen bond (2.96) Hydrogen bond (3.16) Hydrogen bond (3.32) Hydrogen bond (3.05)
Vitexin **ΔG** = -6.8 kcal/mol	Tyr507,Pro505, Thr425,**Trp420,** Leu427,**Trp529,** Ile442,**Phe442, Leu441**	Hydrophobic contact
	Tyr613	Hydrogen bond (2.95) (3.23)
Caffeic acid **ΔG** = -6.1 kcal/mol	**Leu441,**Thr425, Ile442,Leu427, **Trp529,Phe442,** Pro505	Hydrophobic contact
	Tyr613	Hydrogen bond (2.72) (2.84)
P-coumaric acid **ΔG** = -6.0 kcal/mol	Gly436,**Phe437,** Phe447,Ala440, Pro619,Gly559, Met555	Hydrophobic contact
	Thr435 Trp616 Thr561 His617	Hydrogen bond (2.99) Hydrogen bond (2.84) Hydrogen bond (2.99) Hydrogen bond (3.02)
IFN-α **ΔG** = -5.9 kcal/mol	Phe447, Pro619, **Phe437,** Trp616, Met555, Thr435, Gly559, Thr558, Leu443, Phe560	Hydrophobic contact

It is shown that the isovitexin is predicted to interact with E2 protein at residues Tyr618, Trp616, Pro619, Met555, Phe447, Phe437, Ala440, His445, Gly436, Thr435 through hydrophobic interactions, and Thr561 through hydrogen bonds (Table 1, Figure 2, and Figure 3). Isovitexin is a bioactive compound with the lowest affinity value against E2 protein. The result suggested that the isovectin have greatest possible to inhibit the E2 activity due to its binding potency to the target protein [28]. Interestingly, this compound is flavone C-glycosyl compounds known as antioxidants [29]. Isovitexin interacts with the E2 active site at Phe437 through hydrophobic contact and hydrogen bond interactions. The distance between the donor-acceptor hydrogen bond formed with Thr561 is 3.00, indicating that the bond is moderately interacting, so it is assumed that it only acts as a supporting bond. The hydrogen bond has crucial role in maintanance the interaction betweem the protein and ligand. The appearance of hydrogen bond in protein and ligand interaction indicates that the interaction have strong binding to each other. However, this compound only interacts with one active site residue of the E2 protein, which binds to the CD81 protein, so it is suspected that the chance of interaction and inhibition of the formation of the HCV E2-CD81 complex is lower than other compounds that have interactions with more active site residues. In vivo research has shown that isovitexin can protect against liver injury by attenuating histopathological changes and hepatic and serum TNF-α production and improving histopathological structure in a mouse model CCl4-induced liver damage [30].

Orientin is a natural flavonoid that has antioxidant [31], antiviral [32], anti-inflammatory, and cardioprotective activities [33] that have the second-lowest affinity value (-6.9 kcal/mol) is predicted to interact with E2's active site at residue Trp420 through hydrophobic interactions. Another type of interaction between the orientin-protein E2 is the hydrogen bond interaction. The O9 atom in the orientin group binds to Gly517 and Ile538 with H-bond distances of 3.16 and 3.05, respectively. On the other, O10 atoms bond with Thr519 and Asn541 with H-bond distances of 3.32 and 2.96, respectively. The value of the H-bond distance showed a moderate interaction, except for the Thr519 residue, which showed a weak interaction.

Vitexin is a C-glycosylated flavone with known hepatoprotective activity [34], antioxidant, anti-inflammatory, anticancer, neuron protection, and cardio-protection [35], that is estimated to interact with E2 protein with an affinity value of -6.8 kcal/mol [29]. The interaction of vitexin with HCV E2 protein occurs at residues Leu441, Phe442, Trp529, and Trp420, where the last-mentioned residues also interact with orientin via hydrophobic interactions. This compound could possibly inhibit the activity of E2 protein by interfering with the interaction of E2 protein and CD81 as the HCV E2 protein receptor. Vitexin also binds to one residue via H-bond interactions.

Caffeic acid is a polyphenolic compound that has antibacterial, antiviral [36], antioxidant [37], anti-inflammatory [38], immunostimulatory activity [39], hepatoprotective activity [40], and anti-hepatocellular carcinoma [41] estimated to have the fourth-lowest affinity value (-6.1 kcal/mol). This compound interacts with E2's active site at Leu441, Phe442, and Trp529, binding sites for the CD81 residue through hydrophobic interactions. *In vitro*

studies suggest that caffeic acid inhibits the early stages of HCV infection between virion entry and RNA genome translation [42].

P-coumaric acid is a phenolic acid with low toxicity that has antioxidant, anticancer, antimicrobial, antiviral, and anti-inflammatory activities and has mitigating effects on diabetes [43]. It has an affinity value of -6.0 kcal/mol and interacts with a critical active site residue (Trp437) through hydrophobic interactions. *In vitro* studies revealed that p-coumaric acid protects against AAP-induced hepatotoxicity and suppresses hepatic apoptosis [44]. IFN-α as a control had the highest binding affinity of the five potential compounds (-5.9 kcal/mol), which binds to residues Phe447, Pro619, Trp616, Phe437, Thr435, Phe560, Met555, Gly559 through hydrophobic interactions (Table 1, Figure 2, and Figure 3).

The compounds assessed as having the most potential HCV E2 protein fusion inhibitors were vitexin and caffeic acid. The affinity values for these compounds were -6.8 kcal/mol and -6.1 kcal/mol, respectively. These compounds had an affinity value under isovitexin compounds with the lowest affinity values compared to other potential compounds. However, the compounds vitexin and caffeic acid interact more with the crucial residue binding site protein HCV E2 so the disruptive effect caused by interfering with the interaction between the HCV E2-CD81 protein may be greater than that of other compounds.

The interaction between potential compounds and HCV E2 protein is thought to inhibit the process of forming the HCV E2-CD81 protein complex. The formation of this protein complex will initiate the fusion process of HCV with its target cells through the clathrin-mediated endocytosis pathway. In this process, the interaction between the HCV E2-CD81 protein activates the AP2 protein, which plays a role in the vesicle coating process [8]. Clathrin will bind to AP2 and mediate the internalization process of viral particles [45]. If the formation of the HCV E2-CD81 protein complex is disrupted, which results in CD81 not recognizing the HCV E2 protein, the HCV fusion mechanism will be disrupted. With the disruption of the fusion process, the number of internalized viruses is less so that it causes a reduced viral load and reduces the severity of hepatitis C disease.

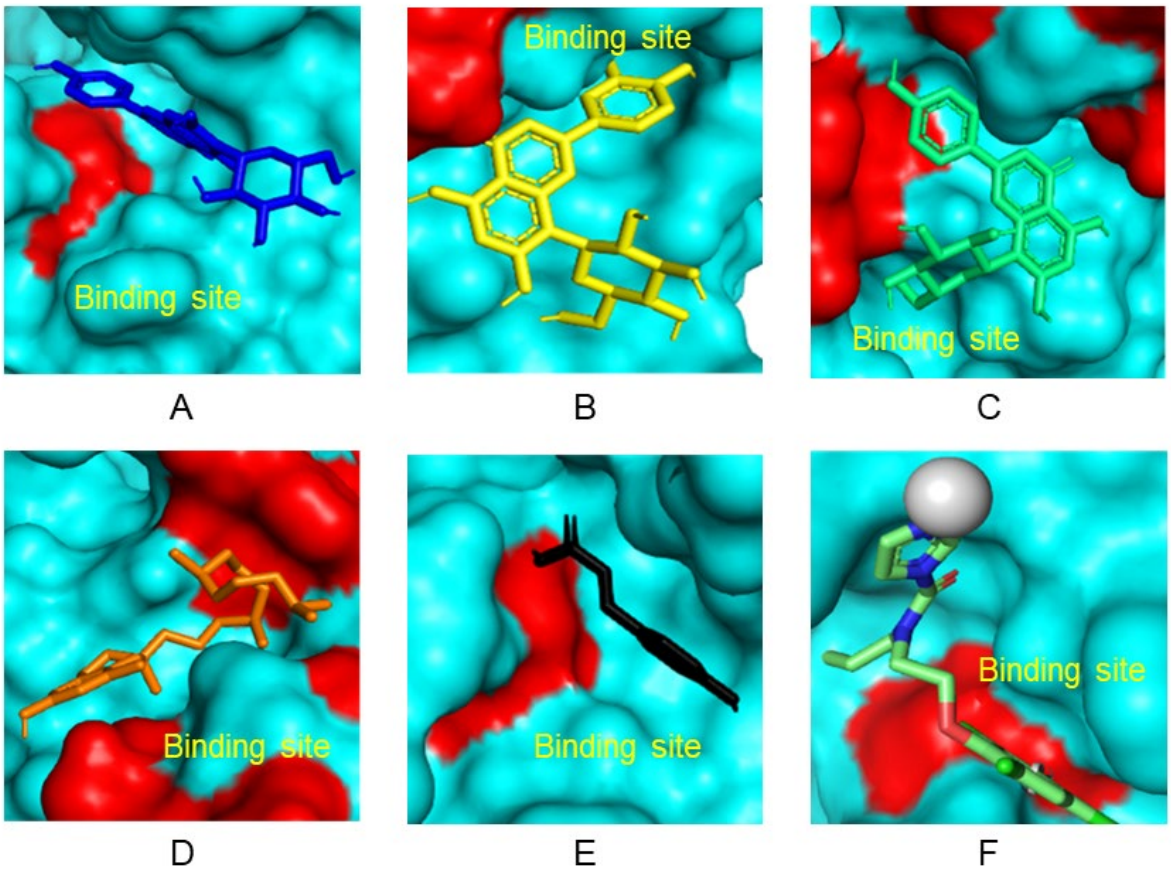

Figure 2. 3D visualization of the top and control bioactive compounds with E2. A) isovitexin, B) orientin, C) vitexin, D) caffeic acid E) p-coumaric acid F) IFNα (control). The red color represents the CD81 binding site of E2.

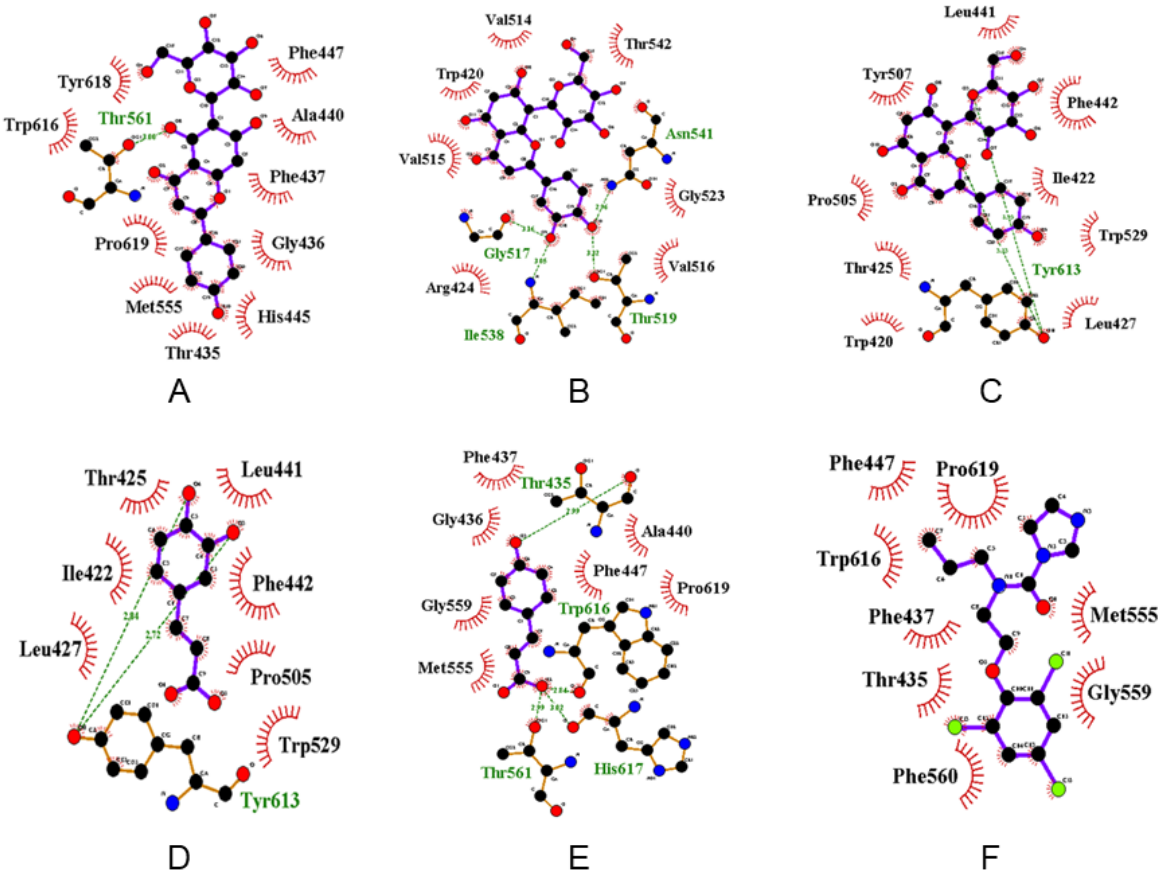

Figure 3. 2D visualization of the top and control bioactive compounds with HCV E2 protein. A) isovitexin, B) orientin, C) vitexin, D) caffeic acid E) p-coumaric acid F) IFN-α (control).

CONCLUSION

The results of the *V. radiata in silico* test against the HCV E2 protein showed the presence of five bioactive compounds that were predicted to act as potential inhibitors of the activity of HCV E2 protein. The results showed that the five bioactive compounds including isovitexin, orientin, vitexin, caffeic acid, and p-coumaric had lower affinity values than IFN- α as a control. Potential compounds are predicted to bind to the CD81 binding site of E2 so that it is possible to inhibit the fusion and internalization of the HCV virus into liver cells and can prevent infection or reinfection of host cells.

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CONFLICT OF INTEREST

The authors declare there is no conflict of interest in this study.

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