PHOTOCHEMISTRY
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Phytochemicals As a Potential Inhibitor of COVID-19: An *In-Silico* Perspective

Rasheed M. A. Q. Jamhour[a], **Afaf H. Al-Nadaf**[b], **Fadel Wedian**[c], **Ghassab M. Al-Mazaideh**[d,*],
Morad Mustafa[e], **Mohammed Ayed Huneif**[f], **Sabry Younis Mahmoud**[g,h], **Eman Saleh Farrag**[i,j],
Fuad Al-Rimawi[k], **Haya Ayyal Salman**[l], **Ali Abdallah Alqudah**[m], and **Fadi Alakhras**[n,**]

[a] *Department of Chemistry and Chemical Technology, Faculty of Science, Tafila Technical University, Tafila, 66110 Jordan*
[b] *Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Mu'tah University, Alkarak, Jordan*
[c] *Department of Chemistry, Faculty of Science, Yarmouk University, Irbid, 22163 Jordan*
[d] *Department of Pharmaceutical Chemistry, College of Pharmacy, University of Hafr Al Batin, Hafr Al Batin, Saudi Arabia*
[e] *Department of Chemistry, School of Science, University of Jordan, Amman, 11942 Jordan*
[f] *Department of Pediatrics, College of Medicine, Najran University, Najran, Saudi Arabia*
[g] *Biology Department, College of Science, University of Hafr Al Batin, Hafr Al Batin, Saudi Arabia*
[h] *Microbiology Department, Sohag University, Sohag, Egypt*
[i] *Clinical Laboratory Science Department, College of Applied Medical Science, University of Hafr Al Batin,
Hafr Al Batin, Saudi Arabia*
[j] *Microbiology Department, South Valley University, Qena, Egypt*
[k] *Department of Chemistry, Faculty of Science and Technology, Al-Quds University, P.O. Box 20002, Jerusalem, Palestine*
[l] *School of Biological Sciences, Universiti Sains Malaysia, Penang, 11800 Malaysia*
[m] *Department of Applied Biology, Faculty of Science, Tafila Technical University, Tafila, Jordan*
[n] *Department of Chemistry, College of Science, Imam Abdulrahman Bin Faisal University, Dammam, Saudi Arabia*
* e-mail: gmazaideh@uhb.edu.sa
** e-mail: fadialakhras@gmail.com
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Abstract—The current research has centered on the use of pharmacological and binding affinity methods to test the 36 compounds as bioactive constituents' inhibitors for COVID-19. Six compounds out of 36 phytoconstituents (rutin, quercetin, catechin gallate, rhamnetin, campesterol and stigmasterol) have demonstrated outstanding molecular docking and drug-like properties as HIV inhibitors Lopinavir and Indinavir. Interestingly, the lowest binding energies (LBE) and the inhibition constant (K_i) have showed that these compounds are able to bind to the P-glycoprotein substrate of 3CL^{pro} and Nsp15. Interestingly, rutin has been found to be an excellent potential inhibitor for COVID-19 proteins because it has the best LBE score and K_i value than those of other compounds, and of its ability to form strong H-bonds with COVID-19 proteins. The compounds that come next to the rutin compound are stigmasterol and campesterol. As a result, these compounds are considered possible novel inhibitors of COVID-19. In order to validate the computational results, more in vitro and in vivo investigations are required to support the findings of this research.

INTRODUCTION

Phytochemicals and secondary metabolites from medicinal plants have activity against COVID-19 as many other plants, which have shown promising therapeutic potential activity against SARS-CoV and SARS-CoV-2 [1–5]. Phytochemicals as well as some medicinal plants with approved antiviral, antimicrobial, and antifungal agents have been exploited towards the development of prophylactic and therapeutic agents for COVID-19 [6–8]. Until this date, there is no specific treatment for COVID-19; therefore, researchers have been directed to test medicinal herbs and phytochemicals as antiviral and anti-corona agents [9, 10]. Currently, antiviral treatments shift toward plant-derived products because they are generally recognized as safe and have less potential for resistance development [11].

The medicinal plants and phytochemicals target multiple proinflammatory and oxidative mediators such as tumor necrosis factor-α (TNF-α) [12]. Among others and because of the involvement of inflammation in the pathogenesis of lung injury, phytochemicals are good candidates as novel compounds in combating corona viruses [13]. In a recent study [10], many compounds isolated from medicinal plants by GC/MS and HPLC such as gallic acid, quercetin, naringin, capsaicin, and psychotrine are important sources for novel antiviral drugs targeting COVID-19. In another study [14], quercetin, kaempferol, myricetin, apigenin, and resveratrol have shown prominent activities against coronaviruses. Alkaloids have also shown antiviral effects against coronaviruses such as Lycorine, which is an indolizidine alkaloid isolated from Lycoris radiata (L'Hér) Herb [8].

Proteins identified in SARS-CoV-2, such as nonstructural protein 15 (Nsp15) [15] and chymotrypsin-like protease (3CL^{pro}) [16], have been targeted by potential medications. Nsp15 is significant in viral replication as indicated with the investigation of a Human CoV 229E [17]. Nsp15 is an endo-ribonuclease that separates 3' of uridylates through a ribonuclease, and this finding can help increasing powerful medications against COVID-19. On the other hand, 3CL^{pro} plays an important role in processing of translated polyproteins. Upon exposure to SARS-CoV-2, several chemical and biological processes occur in the human body [18], and several small molecule medications that counteract corona virus have been surveyed [18].

Nelfinavir has been reported as the best potential compounds against COVID-19 based on docking results [19]. In addition, remdesivir has been reported as one of the 100 inhibitors that inhibit viral replication of SARS-CoV-2 [20, 21]. Furthermore, disulfiram and neratinib as putative covalent inhibitors of SARS-CoV-2 virus 3CL^{pro} [22].

The present study aimed to test many phytoconsitutents by docking technique using Autodock software with different computational methods. This study will add new values in the field of finding new promising inhibitors.

MATERIALS AND METHODS

Protein Preparation

Two human crystal structures of COVID-19 were used from the Protein Data Bank database, namely, 3CL^{pro}(PDB ID:6LU7) [16] and Nsp15 (PDB ID: 6VWW) [15]. The heteroatoms and water molecules were discarded by Bioviadiscovery studio visualizer [23].

Ligands Preparation

Based on clinical studies and literature reviews, 36 of medicinal plant components (flavonoids and alkaloids), which possess antiviral activity, were selected to study the potential binding affinity in the specific binding sites of 3CL^{pro} and Nsp15 of COVID-19. The compounds were rutin, quercetin, apigenin, morin, chrysin, tangeretin, hesperetin, kaempferol, lycorine, curcumin, catechin gallate, myricetin, luteolin, thymoquinone, clarithromycin, ivermectin, spiramycin, praziquantel, mebendazole, β-caryophyllene, vanillin, maslinic acid, bicornin, tannic acid, methyl salicylate, eugenin, kaempferol, rhamnetin, eugenitin, terpenoid taxol, oleanolic acid, stigmasterol, campesterol, zingiberene, δ-cadinene, and humulones.

Several active compounds of the medicinal plants were obtained via Dr. Duke's Phytochemical and Ethnobotanical Databases [24]. All the compounds were undergone a minimization process using Avogadro software [25] with MMFF94 force field, and were saved in PDB format. Gasteiger charges were assigned for all ligands and inhibitors, and the degree of freedom remained default (the number of active torsional movement for all the ligands were less than 6). Ligand molecules were converted to PDBQT format using AutoDock 4.2.6 software [26, 27].

Molecular Docking

This part was achieved by using AutoDock 4.2.6 software, where all rotatable bonds of the compounds were set randomized as completely flexible during the simulation process. Polar hydrogens and Kollman charges were added to 3CL^{pro} and Nsp15 and saved as PDBQT. Grid box size was set to $50 \times 50 \times 50$ points for the active binding sites. The coordinates (x, y, and z, respectively) of the 3CL^{pro} binding site were -10.2439, 17.966, and 66.5084, and of the Nsp15 binding site were -94.65, 19.58, and -28.99 [27, 28]. A maximum number of 100 runs were chosen for each independent Lamarckian genetic algorithm. The remaining parameters were kept default. AutoDock 4.2.6 was used to simulate the docking process. The 2D and 3D potential were visualized and analyzed by the Discovery Studio Visualizer 19.

RESULTS

It has been reported that the key amino acids in the active binding site of 3CL^{pro} are HIS41 and CYS145, whereas in Nsp15 is THR341 [29−33]. Accordingly, there is broad consensus among researchers that

promising new antiviral activity drugs need to interact with these key amino acids to stop protein activity in viral replication.

Here, the molecular docking analysis has been carried out to evaluate the interaction of medicinal plant components (flavonoids and alkaloids) with the two target SARS-CoV-2 proteins (3CL^{pro} and Nsp15). The binding affinity of the interacting compounds to the active site residues (CYS145 and HIS41) of 3CL^{pro} and THR341 of Nsp15 are shown in Table 1.

All the selected ligands docked in 3CL^{pro} and Nsp15 pockets possess varying scores with the enclosed amino acids according to the determined coordinates. Remarkably, as shown Table 1, rutin, quercetin, catechin gallate, rhamnetin, stigmasterol, campesterol overtake the rest of the drugs by forming a strong interaction with the protease of both enzymes with lowest binding energy (LBE) scores and lowest K_i values. Obviously, the affinity of binding of the campesterol has the least value as compared to the values of all selected drugs with 3CL^{pro}, whereas rutin has the least value as compared to the values of all selected drugs with Nsp15.

DISCUSSION

The docking simulations have been carried out for all the selected ligands with two COVID-19 proteins (see Table 1). The findings of this study show that some of phytoconstituent compounds tend to the enzymes more than other compounds. The lowest binding energy (LBE) scores of drugs that are lower than −7.46 kcal/mol with Nsp15 are assumed to show strong interactions and may significantly impair enzymatic activities. For instance, six phytoconstituent compounds (rutin, quercetin, catechin gallate, rhamnetin, stigmasterol and campesterol) have lower LBE scores than −7.46 kcal/mol for Nsp15 and −5.76 kcal/mol for 3CL^{pro}. These compounds are predicted to have the highest potency with strongest interaction in terms of LBE scores and lowest inhibition intensity K_i than the other standard compounds. In addition, these compounds reveal a strong binding affinity to both proteins and their LBE scores are lower than the LBE score of vitamin D, which is considered a potential inhibitor [34]. Amazingly, our pharmacokinetic prediction results have shown that rutin, quercetin, catechin gallate, stigmasterol and campesterol have excellent analysis (LBE and K_i), which are better than the LBE and K_i of the standard HIV-inhibitors (lopinavir and indinavir).The approved antiparasitic drugs by Food and Drug Administration (FDA), such as, ivermectin, spiramycin and praziquantel show good LBE scores and K_i values for Nsp15 and weak interaction with 3CL^{pro} as compared with other phytoconstituents compounds,

which are not approved by the FDA for the treatment of any viral infection [35, 36]. The docked structures of rutin, quercetin, catechin gallate, rhamnetin, stigmasterol, and control complexes of HIV-inhibitors (lopinavir and indinavir) with main protease 3CL^{pro} and endoribonuclease of SARS-CoV-2 are presented in Figs. 1 and 2, respectively, to recognize key amino acid interactions in the pockets and to evaluate the inhibitory effects in the viral replications.

Strikingly, the findings of this study have exhibited the ability of the rutin, quercetin, catechin gallate, rhamnetin, campesterol and stigmasterol to form strong H-bonds with the key amino acids of the main protease 3CL^{pro} (HIS41 and/or CYS145) from the hydroxy group of each compound in the following order: stigmasterol > campesterol > quercetin > rutin > rhamnetin > catechin gallate. Furthermore, for each potential inhibitor, H-bonds with the key residue THR341 in the Nsp15 are found to be in the following order: rutin > campesterol > stigmasterol > catechin gallate > rhamnetin > quercetin.

Interestingly, compared with standard control, the strongest potential natural compounds for both enzymes are found to be in the following order: rutin > stigmasterol > campesterol. This observation indicates that these compounds may be potent drugs to inhibit the viral replication of COVID-19 by halting the activity of the two essential proteins.

CONCLUSIONS

LBE scores, K_i values, and the interactions of all compounds with the active sites of 3CL^{pro} and Nsp15have been measured to confirm the affinity of these compounds to interact with these proteins, and to classify the potential lead drugs according to their affinity and pharmacological properties. Docking scores have revealed that rutin, quercetin, catechin gallate, rhamnetin, campesterol, and stigmasterol have lower LBE score than −7.46 kcal/mol for Nsp15 and −5.76 kcal/mol for 3CL^{pro}. Moreover, the analysis of the interactions of these compounds with the key amino acids in 3CL^{pro} (HIS41 and/or CYS145) and Nsp15 (THR341) has shown that rutin has the best LBE score and K_i value compared with the LBE score and K_i value of other compounds, due to it stability to form strong H-bonds with proteins. The compounds that come next to the rutin compound are stigmasterol and campesterol. In order to confirm the computational findings in this study, the results of this study need further in vitro and in vivo investigations.

Table 1. Lowest binding energy (LBE) scores (kcal/mol) and K_i (µM) values for the phytoconstituents of the selected flavonoids and alkaloids with 3CL^{pro} and Nsp15

| Group	No.	Phytoconstituents	6LU7		6VWW	
			LBE score	K_i	LBE score	K_i
Phytoconstituents of the selected flavonoids and alkaloids	1	Rutin	−5.82	53.88	−8.68	0.43
	2	Quercetin	−5.88	48.77	−7.46	3.41
	3	Apigenin	−6.71	12.09	−6.92	8.45
	4	Morin	−5.59	79.87	−6.92	8.47
	5	Chrysin	−5.85	51.82	−6.37	21.50
	6	Tangeretin	−5.48	96.01	−6.56	15.53
	7	Hesperetin	−4.83	290.44	−6.85	9.57
	8	Kaempferol	−5.79	57.34	−6.77	10.93
	9	Lycorine	−5.45	100.80	−6.85	9.60
	10	Curcumin	−5.66	71.38	−7.54	2.97
	11	Catechin gallate	−5.76	59.95	−7.95	1.49
	12	Myricetin	−5.77	58.70	−7.58	2.78
	13	Luteolin	−6.35	22.23	−6.70	12.30
	14	Thymoquinone	−4.45	548.04	−5.18	159.85
	15	Clarithromycin	−2.23	2304	−7.26	4.74
	16	Ivermectin	−4.99	218.32	−8.08	1.19
	17	Spiramycin	−3.67	2050	−8.64	466.34
	18	Praziquantel	−5.71	65.47	−7.11	6.16
	19	Mebendazole	−6.66	13.14	−7.79	1.94
	20	β-Caryophyllene	−5.51	91.11	−6.96	7.90
	21	Vanillin	−3.98	1210	−4.68	374.19
	22	Maslinic acid	−4.66	385.95	−8.31	0.817
	23	Bicornin	−2.08	3004.0	−6.73	11.76
	24	Tannic acid	+417	n*	+4.02	n*
	25	Methyl salicylate	−3.98	1210.0	−5.56	84.18
	26	Eugenin	−4.97	227.72	−5.48	95.72
	27	Kaempferol	−5.79	57.02	−6.76	11.03
	28	Rhamnetin	−5.86	50.28	−7.67	2.37
	29	Eugenitin	−4.83	289.48	−5.52	89.77
	30	Erpenoidtaxol	−4.19	844.46	−8.04	1.27
	31	Oleanolic acid	−5.25	142.98	−8.50	0.583
	32	Stigmasterol	−6.30	24.08	−8.22	0.942
	33	Campesterol	−5.97	42.20	−8.57	523.83
	34	Zingiberene	−4.96	230.05	−5.78	57.73
	35	δ-Cadinene	−5.28	134.82	−5.80	56.06
	36	Humulones	−6.11	33.23	−7.72	2.18
HIV Inhibitors	1	Lopinavir	−7.79	1.94	−7.86	1.89
	2	Indinavir	−8.12	1.12	−7.09	7.04

* n: no activity.
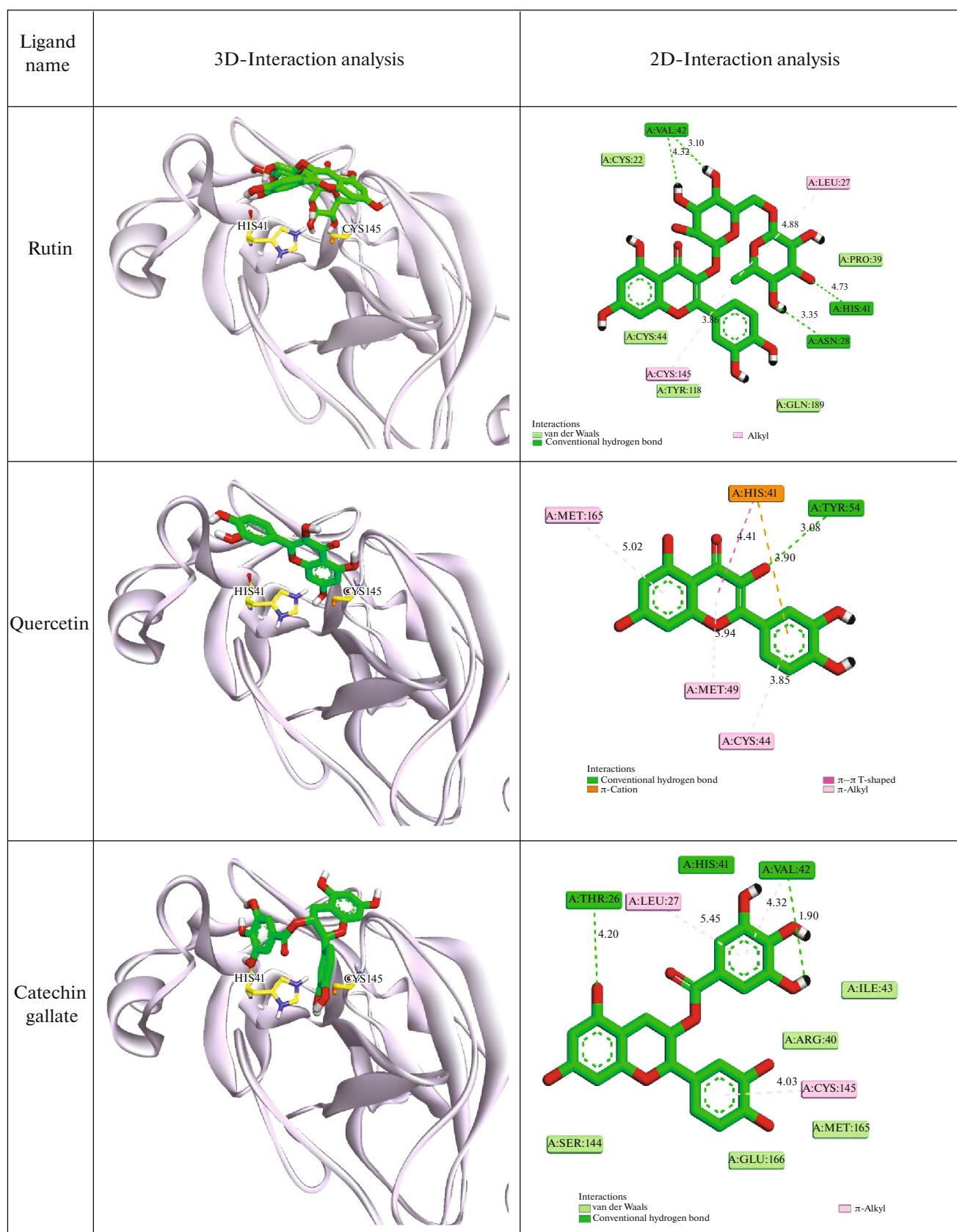
Fig. 1. 2D and 3D interactions analysis of the best scores out of the 36 selected flavonoids and alkaloids compounds with 3CL^{pro}.
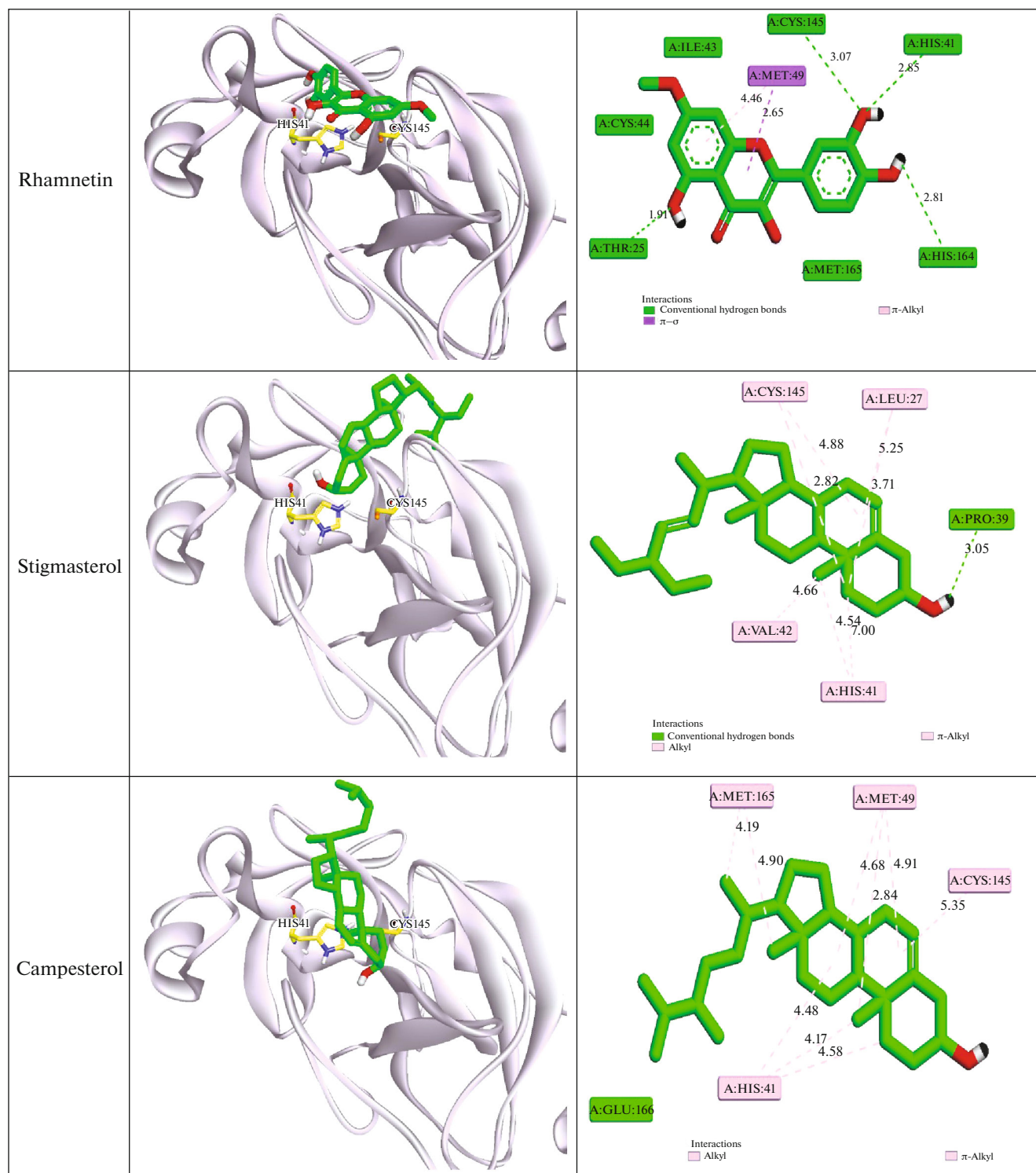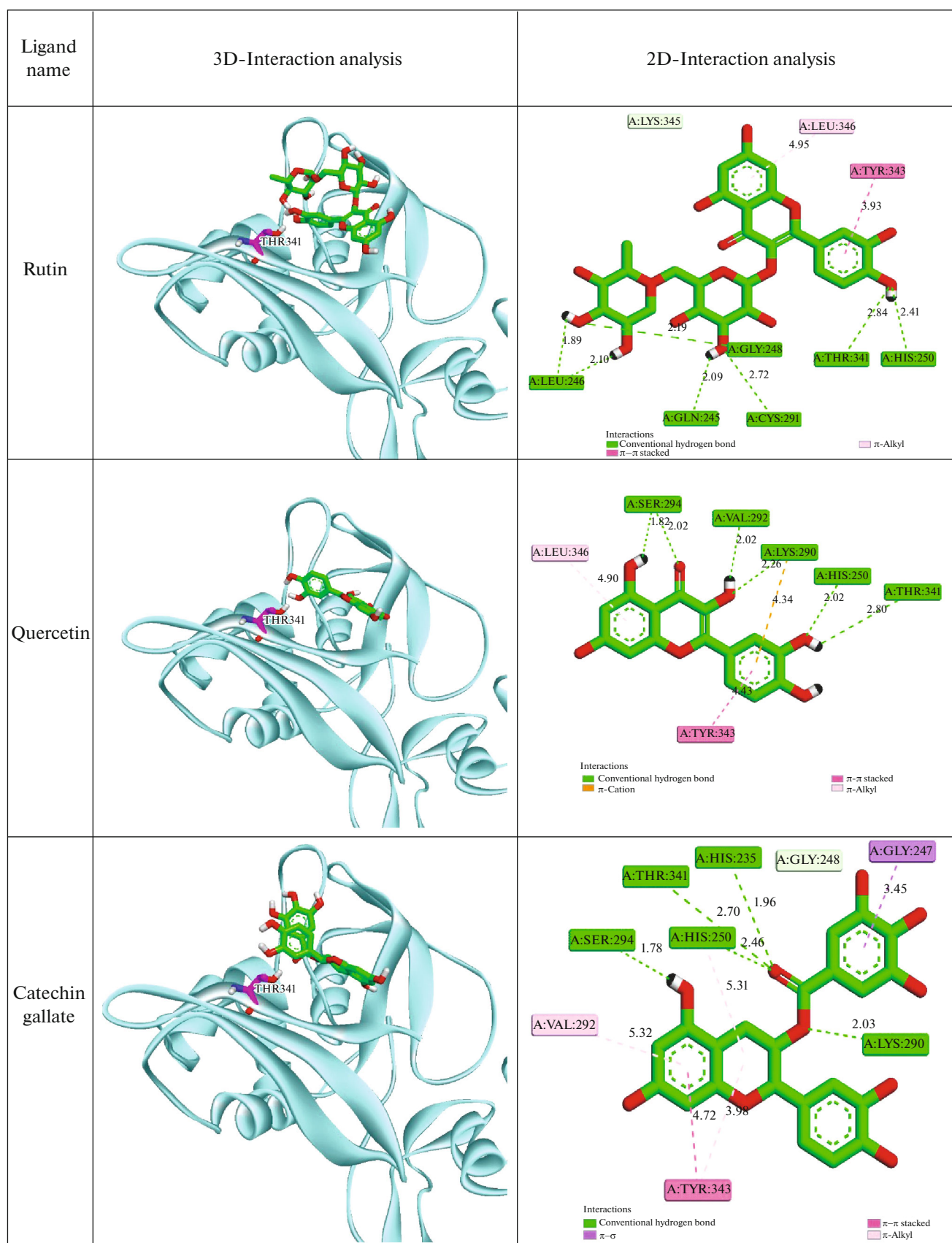
Rhamnetin		
Stigmasterol		
Campesterol		

Fig. 1. (Contd.)

Ligand name	3D-Interaction analysis	2D-Interaction analysis
Rutin		
Quercetin		
Catechin gallate		

Fig. 2. 2D and 3D interactions analysis of the best scores out of the 36 selected flavonoids and alkaloids compounds with Nsp15.

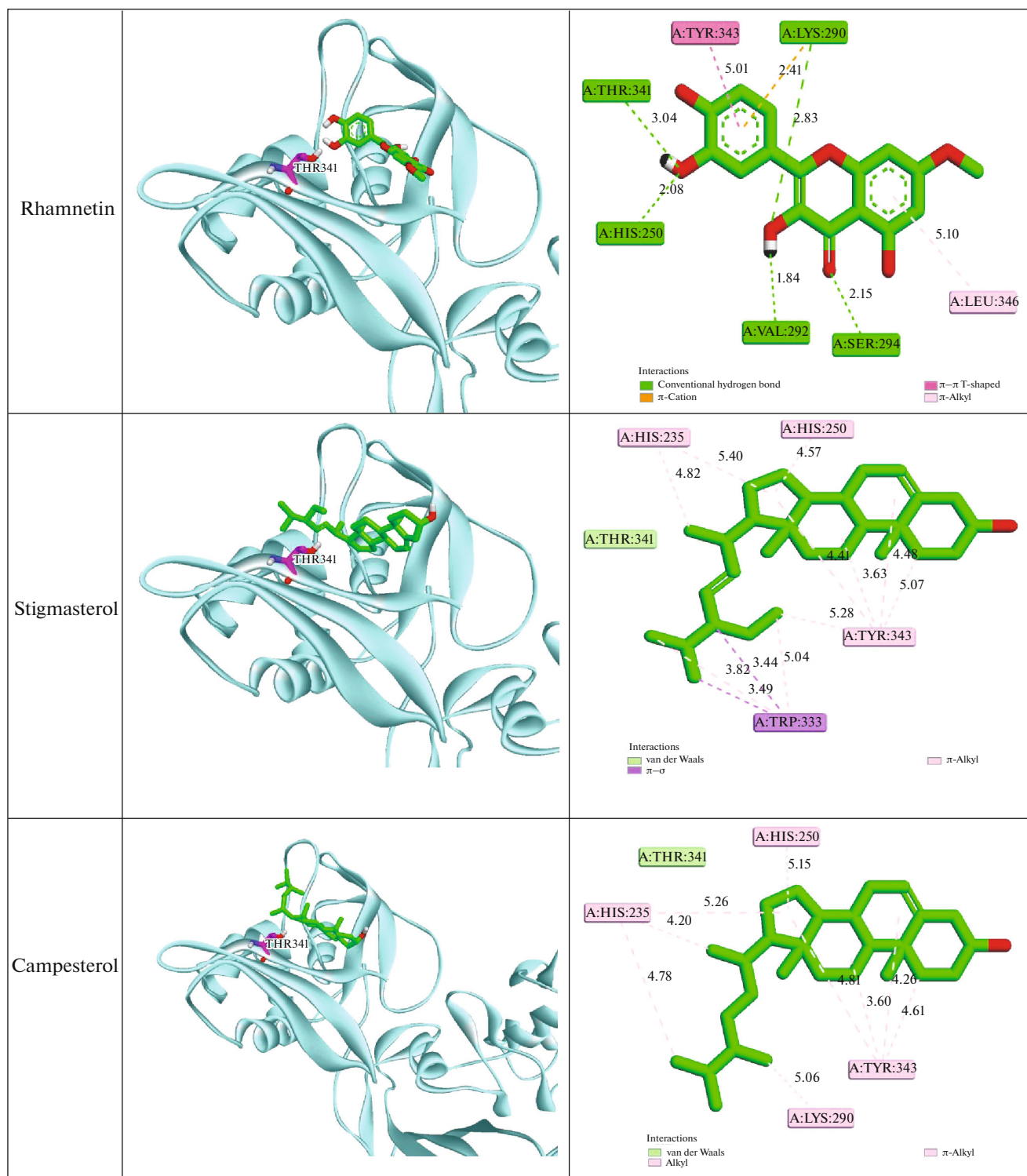|	
Rhamnetin	
Stigmasterol	
Campesterol	

Fig. 2. (Contd.)

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

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