Trop J Nat Prod Res, November 2023; 7(11):5155-5163 **ISSN 2616-0684 (Print)**
ISSN 2616-0692 (Electronic)

Tropical Journal of Natural Product Research

Available online at https://www.tjnpr.org

Original Research Article

Molecular Docking and Pharmacokinetics Studies of *Syzygium aromaticum* Compounds as Potential SARS-CoV-2 Main Protease Inhibitors

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ARTICLE INFO

Article history:
Received 22 August 2023
Revised 09 October 2023
Accepted 19 October 2023
Published online 01 December 2023

ABSTRACT

The current outbreak of the novel coronavirus disease (COVID-19) caused by severe acute respiratory syndrome 2 (SARS-CoV-2), is the major matter of public health concern given its worldwide impact on human life. Despite the research efforts, no effective drug is available for the treatment of this pandemic so far. In the present study, bioactive compounds derived from *Syzygium aromaticum* were screened for their inhibitor potency against SARS-CoV-2 main protease (Mpro) using molecular docking. The analysis revealed that five out of the twenty phytocompounds tested, namely campesterol, stigmasterol, crategolic acid, oleanolic acid and bicornin displayed the highest binding affinity scores against the target protein (-7.7, -7.9, -8.4, -8.5 and -9.2 kcal/mol, respectively). The drug-like and ADMET (Absorption, Distribution, Metabolism, Excretion and Toxicity) predictions showed that these ligands, except bicornin, fall within the Lipinski's rule of five, and have a good pharmacokinetic profile. Our findings suggest therefore that these natural molecules could be considered as potential therapeutic drugs against SARS-CoV-2.

Keywords: COVID-19, SARS-CoV-2, main protease, *Syzygium aromaticum*, molecular docking, drug-likeness, pharmacokinetics

Introduction

In late December 2019, a severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2), also known as COVID-19, has appeared in Wuhan (China) and spread quickly throughout the world.[1] On March 11, 2020, it has been declared as a global health emergency by the World Health Organization (WHO).[2] As on August 06, 2023, the disease has affected 231 countries with a total of 692,614,848 cases and 6,904,580 deaths confirmed.[3] The characteristic symptoms are breathlessness, fever, cough, muscle pain, headache, fatigue, loss of smell and taste, sore throat and diarrhea.[4,5]

SARS-CoV-2 is an enveloped virus belonging to *Nidovirales* order, *Coronaviridae* family and *Betacoronavirus* genera.[6] It represents the largest positive single-stranded RNA virus (genome around 30 kb),[7] and contains different proteins responsible of its replication and propagation processes, including the main protease (Mpro), also known as 3-chymotrypsin-like protease (3CLpro).[8] This homodimeric structure composed of 360 amino acids, possesses a highly conserved sequence sharing 96% of homology with SARS-CoV-1 Mpro.[9,10] It consists of three domains; domain I (8-101 residues), domain II (102-184 residues) and domain III (201-303 residues). The active site is located in the gap between domains I and II, and comprises the catalytic dyad Cys145 and His41.[11,12]

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Citation: Abdelli W and Hamed D. Molecular Docking and Pharmacokinetics Studies of *Syzygium aromaticum* Compounds as Potential SARS-CoV-2 Main Protease Inhibitors. Trop J Nat Prod Res. 2023; 7(11):5155-5163. http://www.doi.org/10.26538/tjnpr/v7i11.18

Official Journal of Natural Product Research Group, Faculty of Pharmacy, University of Benin, Benin City, Nigeria

Due to its critical function in viral life cycle, Mpro represents a drug key target.[13] In addition, no similitude with human proteases has been reported, which means that its inhibition is more likely to have no or less significant side effects.[14,15] Several antiviral approved drugs have been proposed in emergency as COVID-19 treatments, such as lopinavir/ritonavir, indinavir (anti-HIV), chloroquine (anti-malaria), remdesivir (anti-Ebola), oseltamivir (anti-influenza).[16,17,18] Nevertheless, no drug or vaccine has shown to be specific and/or effective to treat SARS-CoV-2 up to now.[19,20] Another promising approach consists to investigate natural resources including medicinal plants. Many phytochemicals have indeed been reported to possess antiviral properties.[21] Some of them have been found to exhibit an inhibitory activity against SARS-CoV-2 main protease.[22,23]

The present study focused on one of the most used and valuable spices, namely *Syzygium aromaticum*. The species, also named clove, is a tropical evergreen tree native from Indonesia and belonging to Myrtaceae family. It is cultivated for its dried unopened flower buds in India, Sri Lanka, Malaysia, Tanzania, Madagascar and Brazil.[24,25] It finds its application as essential oil, whole or ground buds in food, pharmaceutical, cosmetic or agricultural industries.[24,26] It possesses several potent properties including antimicrobial, antioxidant, anti-inflammatory, antiviral, anticarcinogenic, antidiabetic, athelmintic and analgesic activities.[27,28] Regards to its antiviral activity, the studies showed that some compounds like eugenol could act against herpes simplex virus types 1 and 2 (HSV-1 and HSV-2),[29] influenza A virus (IAV)[30] and ebola virus,[31] moreover biflorin and eugeniin could inhibit dengue virus (DENV).[32] The reported activity against numerous RNA viruses would suggest that *S. aromaticum* could potentialy act against SARS-CoV-2. Therefore, the current study aims to assess *in silico* twenty clove compounds against COVID-19 main protease (PDB ID 6LU7) using molecular docking. The best potential inhibitor candidates are thereafter selected for their drug-likeness and ADMET

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

(Absorption, Distribution, Metabolism, Excretion and Toxicity) predictions.

Materials and Methods

Protein preparation

The 3D crystal structure of SARS-Cov-2 protease (Mpro) (PDB ID : 6LU7) was retrieved from RCSB protein databank (https://www.rcsb.org/).[11] The protein was prepared by removing water molecules and the co-crystallized ligand (N3 inhibitor). Then, by adding polar hydrogen atoms and Kollman charges using AutoDock Tools 1.5.6. The target was converted into PDBQT format for molecular docking analysis.[33]

Ligand preparation

Twenty compounds derived from *Syzygium aromaticum* were selected for their therapeutic properties (antiviral, antibacterial, antifungal, anticancer, antioxidant…etc). The 3D structures were obtained in SDF format from PubChem database (https://pubchem.ncbi.nlm.nih.gov/)[34] and converted to PDB format using PyMol. The ligand molecules were subjected to energy minimization using Avogadro with the MMFF94 force field,[35] and saved as PDBQT format using AutoDock Tools 1.5.6 prior to molecular docking.[36] Two antiviral (anti-HIV) FDA drugs (indinavir and lopinavir) were used as positive control.

Molecular docking

The active sites of the Mpro were determined by Discovery Studio Visualizer to generate the grid box parameters. The dimensions grid center were set to x = -13.053, y = 12.793, z = 69.382, the grid size to 50 x 60 x 60 xyz points, and the spacing to 0.375 Å. AutoDock Vina was used to perform molecular docking[37] with an exhaustiveness set to 8. The receptor-ligands interactions were analyzed in 3D and 2D by Discovery Studio Visualizer.[38]

Drug-likeness and ADMET prediction

All the selected ligand molecules were retrieved in SMILE files from PubChem database (https://pubchem.ncbi.nlm.nih.gov/). Their drug-like properties based on Lipinski's rule of five were calculated using Swiss ADME (http://www.swissadme.ch/),[39] and their ADMET (Absorption, Distribution, Metabolism, Excretion and Toxicity) parameters were evaluated using pkCSM (http://biosig.unimelb.edu.au/pkcsm/prediction).[40] The Lipinski's rule includes the following criteria : molecular weight, number of hydrogen bond donors, number of hydrogen bond acceptors and log P. [41]

Results and Discussion

Molecular docking

Twenty *Syzygium aromaticum* compounds were docked into the binding cleft of SARS-CoV-2 Mpro (6LU7), and compared to the native ligand and two reference drugs. The binding energies and the amino acid residues involved in the interactions are summarized in Table 1. The lowest energy score is considered as the highest binding affinity and therefore, the most favorable binding mode.[42] It was found that four compounds have lower binding energy against Mpro than the native ligand (N3) (-7.7 kcal/mol) namely, stigmasterol, crategolic acid, oleanolic acid and bicornin (-7.9, -8.4, -8.5 and -9.2 kcal/mol, respectively), while four others ranked at or near the N3 energy score, namely campesterol, ellagic acid, kaempferol, quercetin with -7.7, -7.5, -7.6 and -7.5 kcal/mol, respectively. Moreover, oleanolic acid, crategolic acid and bicronin show the highest binding affinity to the target compared to the standard antiviral drugs lopinavir (-8.0 kcal/mol) and indinavir (-8.1 kcal/mol). The lowest docking scores have been noted at -4.9 kcal/mol for both eugenol and vanillin, closely followed by acetyl eugenol, carvacrol and gallic acid with respectively -5.2, -5.3 and -5.5 kcal/mol.

The best ranked binding pose and the target residues involved are presented in Figure 1 and Figure 2. Stigmasterol formed one conventional H-bond with Thr26, two π-alkyl contact with His41 and nine alkyl contacts with Pro168, Leu167, Met165 (2), Cys145 (3), Leu27, Met49. Oleanolic acid formed two conventional H-bonds with Asn142 and Thr190, three π-alkyl contacts with His163 (2) and His172, and three alkyl contacts with Pro168 (2) and Cys145. Crategolic acid had three conventional H-bonds with Asn142 and Thr190 (2), one carbon-H-bond with Pro168, three π- alkyl contacts with His163 (2) and His172, and two alkyl contacts with Cys145 and Pro168. Campesterol developed one conventional H-bond with Met49, one π-sigma contact with His41, three π-alkyl contacts with His41 (2) and His163, and six alkyl contacts with Met49 (2), Cys145 (2), Met165 and Leu141. Whereas bicornin established the most conventional interactions with 6LU7 through thirteen H-bonds with Cys145 (2), Glu166 (4), Thr190 (3), Phe140 (2), His164 and Gln192. It also made one carbon-H-bond with Pro168, four π-alkyl interactions with Leu141, Pro168 and Met165 (2), one π-π T-shaped contact with His41, one π-sulfur contact with Met49 and one π-anion with Glu166.

Table 1: Docking analysis of *Syzygium aromaticum* compounds and reference drugs against SARS-CoV-2 Mpro (6LU7)

Ligand	Binding energy (kcal/mol)	No. of H-bonds	H-bonds interaction residues (Distance Å)	Other interaction residues
Native ligand N3	-7.7	7	Gln189 (2.20, 2.66), Phe140 (2.27), His41 (2.52), His163 (2.21), Glu166 (2.11, 3.67)	Thr25, Met165
β-caryophyllene	-6.1	0	-	3 His41, Met49, 3 Met165, Cys145
Carvacrol	-5.3	1	Glu166 (2.05)	Gln189, 2 His41, 3 Met165
Campesterol	-7.7	1	Met49 (2.20)	3 His41, 2 Met49, 2 Cys145, Met165, Leu141, His163, Gln189
Stigmasterol	-7.9	1	Thr26 (1.74)	Met49, 3 Cys145, 2 Met165, Leu27, Leu167, 2 His41, Pro168
Vanillin	-4.9	8	Cys145 (2.95, 3.94), His163 (1.94), Glu166 (1.77, 3.34), Leu141 (1.96), Ser144 (1.89), Phe140 (3.09)	His163, His172
Oleanolic acid	-8.5	2	Asn142 (2.20), Thr190 (1.78)	2 Pro168, Cys145, 2 His163, His172
Crategolic acid	-8.4	4	Asn142 (1.86), Pro168 (3.72), Thr190 (1.86, 2.35)	Pro168, Cys145, 2 His163, His172, Gln192
Ellagic acid	-7.5	5	Thr190 (1.80, 1.87), Cys145 (2.68), His164 (2.25), Glu166 (2.81)	Gln189, 4 Met165, Arg188

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

Gallic acid	-5.5	4	Gln192 (2.12), Glu166 (2.00), Thr190 (2.09, 2.14)	Met165, Pro168
Bicornin	-9.2	14	Cys145 (2.88, 3.65), Glu166 (1.85, 2.09, 2.16, 2.35), Thr190 (1.97, 2.06, 3.01), Phe140 (1.81, 2.51), His164 (2.72), Gln192 (2.95), Pro168 (2.99)	Glu166, Met49, His41, 2 Met165, Pro168, Leu141, His172, Gln192
Kaempferol	-7.6	4	Thr190 (2.01), Glu166 (1.99), His164 (2.48), Asp187 (2.06)	3 Met165, His41, Pro168
Quercetin	-7.5	6	Tyr54 (2.53), Gln192 (2.54), Thr190 (1.92), Glu166 (1.80), His164 (2.20), Asp187 (2.11)	Gln189, 3 Met165, His41, Pro168
Myricetin	-7.4	6	Tyr54 (2.52), Gln192 (2.55), Thr190 (2.21), Glu166 (1.77), His164 (2.23), Asp187 (2.13)	Gln189, 3 Met165, His41, Pro168
Eugenol	-4.9	3	Glu166 (2.17), Arg188 (3.26), Thr190 (3.71)	Met49, 2 Met165
Eugenin	-6.0	8	Gly143 (1.92), Ser144 (3.04), His163 (1.94), His164 (2.24), Phe140 (3.27), Glu166 (3.77), Cys145 (3.33, 3.37)	His41, His163, His172
Eugenitin	-6.0	2	Glu166 (2.12), Asp187 (3.31)	Gln189, His41, 3 Met165, 2 Met49
Rhamnetin	-7.3	5	Gln192 (2.54), Thr190 (1.91), Glu166 (1.76), His164 (2.20), Met49 (3.61)	Gln189, 3 Met165, His41, Met49, Pro52, Arg188, Pro168
Biflorin	-7.1	10	Cys145 (2.02, 3.67, 3.97), His163 (2.02), Glu166 (2.04, 2.05), Phe140 (2.17), Leu141 (2.59), Asn142 (3.49), Gly143 (2.28)	Leu27, Cys145
Acetyl eugenol	-5.2	2	His41 (2.22), Cys145 (1.94)	Cys145
α-humulene	-5.8	0	-	Met49, 3 Met165, Cys145, 2 His41
Indinavir	-8.1	9	His41 (2.06), Cys145 (3.57), Glu166 (1.76, 2.07, 2.24), Thr190 (2.06), Met165 (2.99), Gln189 (2.84, 3.31)	2 Pro168, His163, Cys145, Ala191, Met165
Lopinavir	-8.0	6	Glu166 (2.51), Met165 (3.00), Arg188 (2.31), Gln189 (1.95, 2.75, 2.89)	Glu166, 2 His41, Pro168, 2 Met165, Leu167, Met49, Leu27, Arg188

The redocked native ligand (N3) formed six conventional H-bonds with Glu166, Gln189 (2), Phe140, His41 and His163, one carbon-H-bond with Glu166, one π-sigma contact with Thr25, and one alkyl contact with Met165. Regarding the positive controls, indinavir showed six conventional H-bonds to 6LU7 at His41, Cys145, Glu166 (3) and Thr190, three carbon-H-bonds at Met165 and Gln189 (2), one alkyl contact at Pro168 and five π-alkyl contacts at His163, Cys145, Pro168, Ala191 and Met165. Lopinavir exhibited four conventional H-bonds at Glu166, Gln189, Met165 and Arg188, two carbon-H-bonds at Gln189, two π-alkyl interactions at His41 and Met165, also five alkyl interactions at Pro168, Met165, Leu167, Met49 and Leu27, one π-π T-shaped contact at His41 and one π-anion contact at Glu166.

Oleanolic acid is a pentacyclic triterpenoid reported to possess antiviral activity against hepatitis C virus (HCV),[43] human immunodeficiency virus (HIV),[44] herpes simplex virus (HSV)[45] and influenza virus (H1N1).[46] Crategolic acid is also a pentacyclic triterpenoid that can act as anti-HIV agent.[47] Stigmasterol and campesterol are phytosterols that have been shown to inhibit influenza virus by exhibiting synergistic effects.[48] Recent computational works have demonstrated the inhibitory potential of the above-mentioned compounds against SARS-CoV-2 main protease (6LU7), which support our findings. Indeed, in a study conducted by Rehman *et al.*[49], crategolic acid, also called maslinic acid, showed only hydrophobic interactions towards Mpro with a binding energy of -8.1 Kcal/mol. This value is found to be slightly higher compared to our result (-8.4 kcal/mol). A docking perfomed by Rangsinth *et al.*[50] showed that oleanolic acid interacted with Mpro at -8.35 kcal/mol with three H-bonds (2Cys145 and Ser144). The binding energy was reported to be lower compared to the native ligand (N3) and the reference drug (lopinavir) tested (-7.97 and -7.41 kcal/mol, respectively); indicating a better affinity with COVID-19 Mpro, which is consistent with our results.

Moreover, Jannat *et al.*[51] docked campesterol and stigmasterol with Mpro. The results showed the respective scores of -7.5 and -7.9 kcal/mol with no H-bond for the first compound and two conventional H-bonds (2Thr26) for the second compound. These docking values are quite similar to our findings (-7.7 for campesterol and -7.9 kcal/mol for stigmasterol).

In the present study, bicornin which is an ellagitannin,[52] exhibited the most significant binding affinity towards SARS-CoV-2 Mpro (-9.2 kcal/mol). The same docking score was obtained by Rehman *et al.*[49], whereas, the compound showed the second best result after rutin (-9.4 kcal/mol). To the best of our knowledge, no inhibitory activity was previously reported for bicornin against other viruses.

Furthermore, it can be observed according to Table 1, that all the clove compounds (except gallic acid and eugenol), as well as the positive controls, bound to either both Cys145 and His41 residues or at least one of them through hydrogen or hydrophobic interactions. These results are in agreement with previous studies.[51,53,54] Cys145 and His41 constitute the catalytic dyad of SARS-Cov-2 Mpro, which is responsible for the proteolytic activity;[55,56] that means that the molecules tested could act as inhibitors of main protease activity. Therefore, *Syzygium aromaticum* can be suggested as potential candidate drug against COVID-19.

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

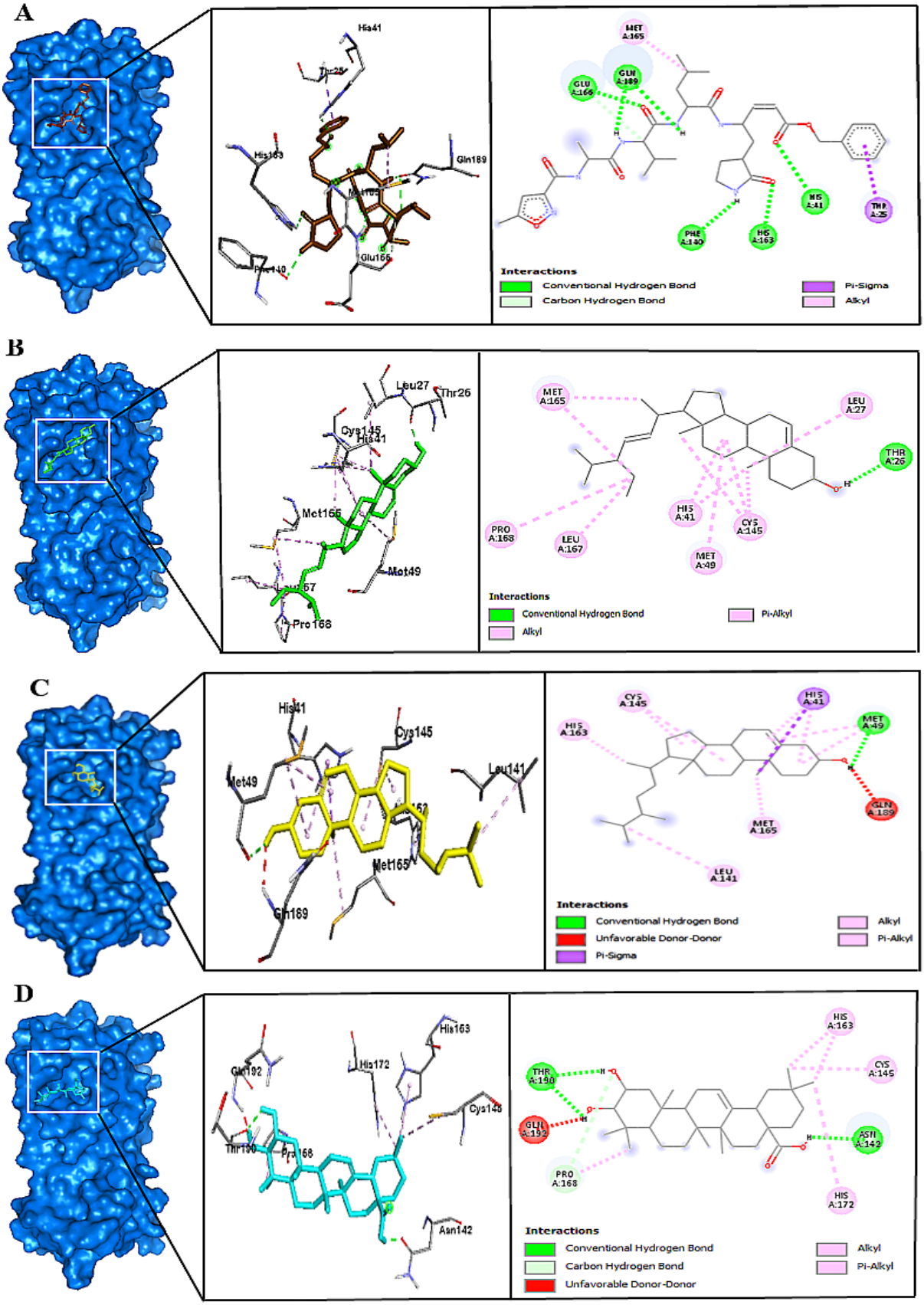

Figure 1: 3D and 2D illustrations of the best docked complexes : (A) native ligand (N3), (B) stigmasterol, (C) campesterol, (D) crategolic acid within the active and catalytic site of SARS-CoV-2 Mpro.

ISSN 2616-0684 (Print)
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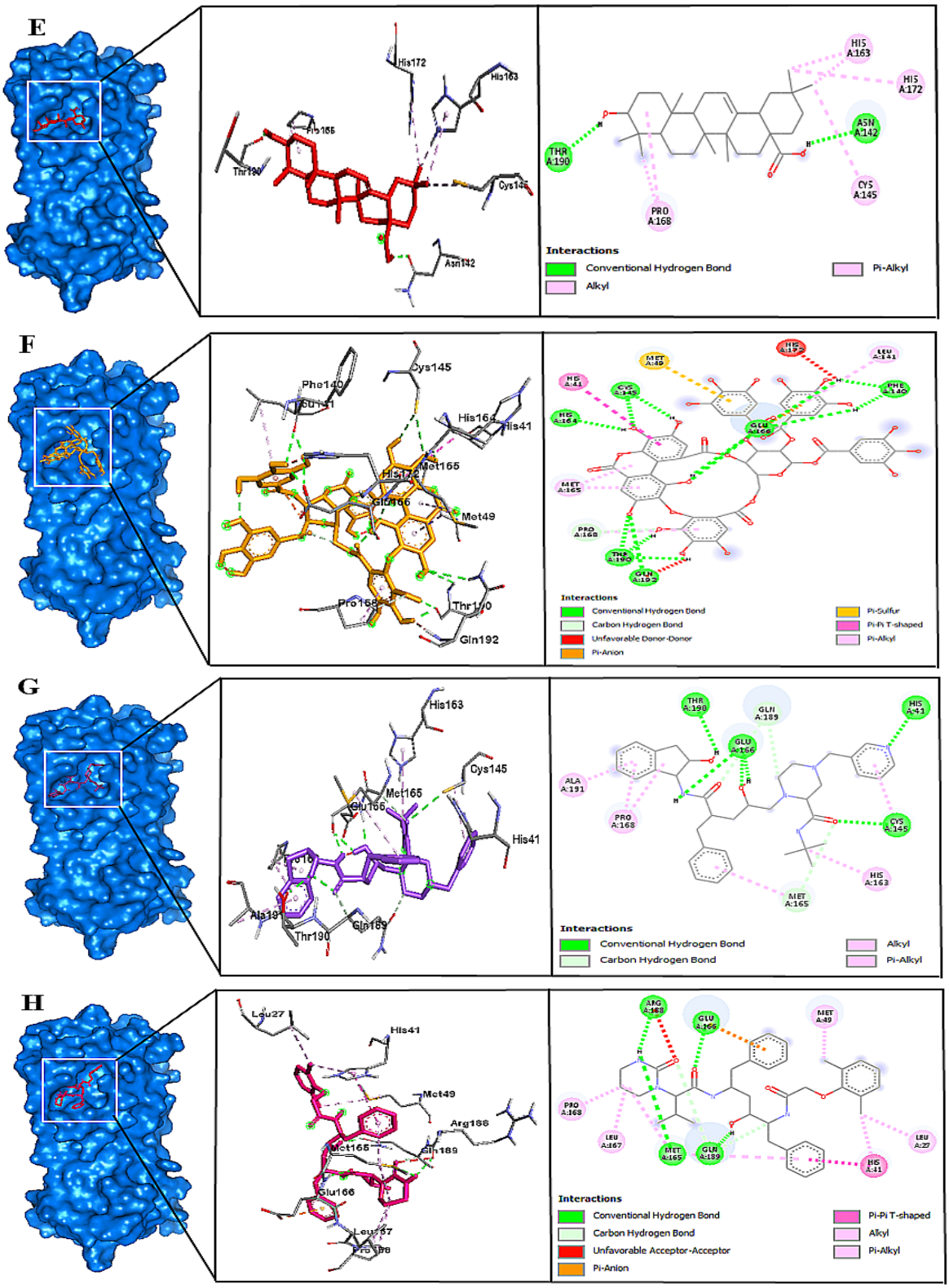

Figure 2: 3D and 2D illustrations of the best docked complexes : (E) oleanolic acid, (F) bicornin, (G) indinavir, (H) lopinavir within the active and catalytic site of SARS-CoV-2 Mpro.

Drug-likeness and ADMET analysis

The ligands molecules with the best docking scores (equal or higher than -7.7 kcal/mol) were selected to study their drug-like nature and pharmacokinetic behavior. The corresponding data are given in Tables 2 and 3. The Lipinski's rule of five states the following criteria : molecular weight (≤ 500 g/mol), octanol-water partition coefficient (logP ≤ 5), number of hydrogen bond donors (≤ 5) and number of hydrogen acceptors (≤ 10). In general, a molecule with no more than one violation is considered as an orally active drug.[57] According to Table 2, all the compounds fulfilled the rule except bicornin (3 violations). This latter showed also the lowest bioavailability score (BS) (0.17) indicating its fail to pass the rule of five.[58] Campesterol, stigmasterol, crategolic acid and oleanolic acid have molecular weights ≤ 500 g/mol; which reflects their ease to be transported, diffused and absorbed by the body compared to bicornin, lopinavir and indinavir,[57,59] whereas, their lipophilicity (logP values) is slightly higher than the optimum. Previous reports suggested nevertheless that these compounds might be good oral drug candidates.[60,61] Regarding

the Veber's rule, which states that the topological polar surface area (TPSA) ≤ 140 Å^2 and the rotatable bonds (RB) ≤ 10, [62] only campesterol, stigmasterol, cratgeolic acid and oleanolic acid meet the two criteria. Thus, the four compounds are more likely to display optimal membrane permeability with good oral bioavailability.[62,63]

Pharmacokinetics properties such as blood-brain barrier (BBB) penetration, aqueous solubility (logS), human intestinal absorption (HIA), cytochrome P450 isoforms inhibition, Caco-2 permeability, hepatotoxicity, oral acute toxicity are crucial parameters in the development of appropriate drug candidate.[64,65] For this study, bicornin has been excluded since it could not satisfy the rule of five. The results summarized in Table 3 showed that only two compounds, namely campesterol and stigmasterol, seem to cross the blood-brain barrier, as revealed by their positive log BB value. In addition, the distribution to the brain can be prompt as these latter are higher than 0.3. [66] The human intestinal absorption (HIA) values indicate that the four phytocompounds can be better absorbed from gastrointestinal tract (more than 90% of probability) than the reference compounds (63.843%, on average). The aqueous solubility (logS) is one of the major property influencing absorption.[39] It can be considered that crategolic acid, oleanolic acid and indinavir are soluble as their logS ranged between -4 and -2, lopinavir is moderately soluble (-4.819), whereas campesterol and stigmasterol are poorly soluble since their logS ranged between -10 and -6. [67]

Another critical parameter related to absorption is the Caco-2 cell line, a monolayer composed of human epithelial colorectal adenocarcinoma cells, recommended to estimate drug permeability through human intestinal epithelium due to its morphological and functional similarities with human enterocytes.[40,68] Based on this model, a compound with an apparent permeability value (log Papp) greater than 0.90 cm/s, has a high Caco-2 permeability.[40] In our case, only oleanolic acid, stigmasterol and campesterol are predicted to have high Caco-2 permeability.

The P-glycoprotein (P-gp) and cytochrome P450 (CYP) are well known to have a significant role in xenobiotic metabolism to protect tissues.[69] It should be noted that if the P-gp and CYP450 activities are inhibited by a drug, this can lead to adverse effects and drug-drug interactions.[70,71] It is found that all test compounds are CYP450 non-inhibitors except for lopinavir and indinavir concerning CYP2C19, CYP2C9 and CYP3A4 isoforms, and P-gp non inhibitors except for campesterol, stigmasterol, lopinavir and indinavir. Moreover, only the reference drugs seem to be P-gp substrates and hence, can be exuded from cells by the latter. Furthermore, the renal organic cation transporter 2 (OCT2) is a protein transporter that plays a vital role in drugs clearance, and gives informations about their potential contraindications.[72] It seems that none of the six test compounds is predicted to be renal OCT2 substrate, suggesting that all of them can be eliminated through OCT2 substrate.[66]

Also according to Table 3, the toxicity study reveals that none of the compounds are mutagenic, and only stigmasterol and campesterol are predicted to not induce hepatotoxic effects. Regarding the oral rat acute toxicity, the median lethal dose (LD50) values range from 890 to 5000 mg/Kg body weight. According to the Globally Harmonized System (GHS),[73] lopinavir and indinavir are classified in category V and the rest of the compounds in category IV, which means that they are slightly toxic but can be considered as safe.[66,74]

Conclusion

Through this study, 18 out of 20 compounds of *Syzygium aromaticum* have been able to interact with the active site of SARS-CoV-2 main protease (6LU7), which comprises the catalytic pair residues Cys145-His41. Five of them (stigmasterol, campestrol, crategolic acid, oleanolic acid and bicornin) have been identified, according to their docking scores, as the best ligands for 6LU7 compared to the native ligand (N3), and therefore, the most interesting to assess for their drug-likeness and ADMET (absorption, metabolism, excretion and toxicity) properties. Only bicornin was found to not obey the Lipinski's rule of five and thus, was discarded from the ADMET study, although it showed the lowest binding energy compared to the nineteen other clove compounds and to the standard drugs tested. Furthermore, the four top phytocompounds displayed overall good druggable features and low toxicity, which leads us to propose them as potential drug candidates against SARS-CoV-2. Further *in vitro* and *in vivo* studies should nevertheless be conducted in order to confirm their antiviral efficacy.

Table 2: Drug-likeness properties of the best ligand molecules

Ligand	Lipinski's rule of five					Veber's rule			BS
	MW	LogP	HBD	HBA	NV	RB	TPSA	NV	
Campesterol	400.68	6.90	1	1	1	5	20.23	0	0.55
Stigmasterol	412.69	6.97	1	1	1	5	20.23	0	0.55
Crategolic acid	472.70	5.24	3	4	1	1	77.76	0	0.56
Oleanolic acid	456.70	6.06	2	3	1	1	57.53	0	0.85
Bicornin	1088.75	0.32	16	30	3	9	503.85	1	0.17
Lopinavir	628.80	4.40	4	5	1	17	120.00	1	0.55
Indinavir	613.79	2.76	4	7	1	14	118.03	1	0.55

MW : Molecular weight (g/mol), LogP : octanol/water partition coefficient, HBD : Number of hydrogen bond donors, HBA : Number of hydrogen bond acceptors, RB : Rotatable bonds, TPSA : Topological polar surface area (Å^2), NV : Number of violations, BS : Bioavailability score

Table 3: Pharmacokinetic properties of the best ligand molecules

Property	Campesterol	Stigmasterol	Crategolic acid	Oleanolic acid	Lopinavir	Indinavir
BBB	0.774	0.771	-0.493	-0.14	-0.83	-0.735
HIA	94.543	94.97	100	99.931	65.607	62.078
LogS	-7.068	-6.682	-3.042	-3.074	-4.819	-3.611
Caco-2	1.223	1.213	0.629	1.17	0.063	0.823
P-gp subsrate	No	No	No	No	Yes	Yes
P-gp inhibitor	Yes	Yes	No	No	Yes	Yes
Renal OCT2 substrate	No	No	No	No	No	No
CYP1A2 inhibitor	No	No	No	No	No	No

CYP2C19 inhibitor	No	No	No	No	Yes	Yes
CYP2C9 inhibitor	No	No	No	No	Yes	Yes
CYP2D6 inhibitor	No	No	No	No	No	No
CYP3A4 inhibitor	No	No	No	No	Yes	Yes
AMES	No	No	No	No	No	No
Hepato-toxicity	No	No	Yes	Yes	Yes	Yes
LD_{50}	890	890	2000	2000	5000	5000

BBB : Blood-brain barrier, HIA : Human intestinal absorption, LogS : Aqueous solubility, Caco-2 : Colorectal adenocarcinoma cells, P-gp : Permeability glycoprotein, CYP : Cytochrome P450, AMES : Ames mutagenicity, LD50 : Oral rat acute toxicity (mg/Kg).

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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