Taylor & Francis
Taylor & Francis Group

Journal of Biomolecular Structure and Dynamics

ISSN: (Print) (Online) Journal homepage: https://www.tandfonline.com/loi/tbsd20

Molecular docking, pharmacophore modelling, MD simulation and *in silico* ADMET study reveals bitter cola constituents as potential inhibitors of SARS-CoV-2 main protease and RNA dependent-RNA polymerase

Abimbola Peter Oluyori, Bolatito Eunice Olanipekun, Oluyomi Stephen Adeyemi, Godshelp Osas Egharevba, Abayomi Emmanuel Adegboyega & Oluwole Solomon Oladeji

To cite this article: Abimbola Peter Oluyori, Bolatito Eunice Olanipekun, Oluyomi Stephen Adeyemi, Godshelp Osas Egharevba, Abayomi Emmanuel Adegboyega & Oluwole Solomon Oladeji (2022): Molecular docking, pharmacophore modelling, MD simulation and *in silico* ADMET study reveals bitter cola constituents as potential inhibitors of SARS-CoV-2 main protease and RNA dependent-RNA polymerase, Journal of Biomolecular Structure and Dynamics, DOI: 10.1080/07391102.2021.2024883

To link to this article: https://doi.org/10.1080/07391102.2021.2024883

Published online: 07 Jan 2022.

Submit your article to this journal

Article views: 157

View related articles

View Crossmark data

Full Terms & Conditions of access and use can be found at
https://www.tandfonline.com/action/journalInformation?journalCode=tbsd20

JOURNAL OF BIOMOLECULAR STRUCTURE AND DYNAMICS
https://doi.org/10.1080/07391102.2021.2024883

Taylor & Francis
Taylor & Francis Group

Check for updates

Molecular docking, pharmacophore modelling, MD simulation and *in silico* ADMET study reveals bitter cola constituents as potential inhibitors of SARS-CoV-2 main protease and RNA dependent-RNA polymerase

Abimbola Peter Oluyori[a,b], Bolatito Eunice Olanipekun[d], Oluyomi Stephen Adeyemi[a,c], Godshelp Osas Egharevba[a,b], Abayomi Emmanuel Adegboyega[e] and Oluwole Solomon Oladeji[a,b]

[a]Good Health and Well-Being Research Group, Landmark University SDG 3, Omu Aran, Nigeria; [b]Department of Physical Sciences, Landmark University, Omu Aran, Nigeria; [c]Department of Biochemistry, Landmark University, Omu Aran, Nigeria; [d]Department of Chemistry and Industrial Chemistry, Kwara State University, Malete, Nigeria; [e]Department of Biochemistry, University of Jos, Jos, Nigeria

Communicated by Ramaswamy H. Sarma

ABSTRACT

A mini survey was employed in the search of herbs and spices which people believe could prevent them from contracting COVID-19. Phytochemicals which have been earlier implicated for the bioactivity of the afore-mentioned herbs and spices were identified through literature search. The phytochemicals were then subjected to pharmacore modelling, molecular docking and molecular dynamics simulation in order to identify phytochemicals that could serve as inhibitors of 3-Chymotryprin-like protease and RNA dependent–RNA polymerase of SARS-CoV-2. The drug-likeness and toxicity profile of the phytochemicals were afterwards predicted via ADMET studies. The mini survey showed ginger, garlic, bitter cola, as the lead-herbs which could find application in anti- COVID-19 therapy. Literature search revealed 27 phytochemicals were implicated for bioactivity of these herbs. Of these 27 phytoconstituents that were docked with 3-chymotrypsin-like protease and RNA dependent–RNA polymerase, the constituents of bitter cola had lower docking scores than other phytochemicals. MD simulation results showed that Garcinia biflavonoid I displayed less comformational changes and the better binding free energy. Also, the garcinia biflavonoids had relatively safe ADMET predictions. Hence, Garcinia biflavonoids and some other constituents of bitter cola could be further modified so as to obtain safe pharmaceutical intervention for the COVID-19 challenge.

ARTICLE HISTORY
Received 3 July 2021
Accepted 18 December 2021

KEYWORDS
Pharmacophore modelling; molecular docking; simulation; ADMET prediction; bitter cola; Garcinia biflavonoids; COVID-19

1. Introduction

Generally, corona viruses are a family of microorganisms which cause illnesses ranging from common cold to severe diseases (WHO, 2021). However, towards the end of 2019, severe acute respiratory syndrome corona virus (SARS-CoV-2), a dangerous and extremely contageous type of the disease originated from Wuhan, China. This was later declared as a pandemic by World Health Organization (WHO, 2021). The emergence of the new class of corona virus has impacted negatively on every sector of the global society (Chakraborty & Maity, 2020). People of all races and professions have been severely affected. Consequently, the economy of several nations have experienced major set-backs with many loosing their jobs and means of livelihood (Pramathesh et al., 2020). The mental health and educational progress of several students were also affected, contributing to youth engagement in societal vices (Adarkwah, 2021; Dwivedi et al., 2020; Omonijo et al., 2013; Sahu, 2020).

As at 30th April, 2021, there has been 149,910,744 confirmed cases and 3,155,168 confirmed deaths resulting from COVID-19 (WHO, 2021). In addition to this alarming death record, some information that was injected into public space resulted in acute fear which in turn affected the mental health of many (Otu et al., 2020). This fear was further fueled by the uncertainty which accompanied several COVID-19 pharmaceutical interventions such remdesivir/hydroxychloroquine (Zou et al., 2020) and the long time it takes to conduct clinical trials for vaccines before their eventual approval and availability for public use (Tripathi et al., 2020).

Furthermore, in response to several beliefs about tradomedical intervention, public awareness has been heightened in order to educate the public about the limitation of herbal medicines in the treatment of corona virus (Ang et al., 2020; López-Alcalde et al., 2020). However, certain herbal remedies contains various secondary metabolites which have been earlier reported as modulators of the immune systems in preventive medicine (Trinh et al., 2020; Wink et al., 2012).

Hence, in the search for molecules which can prevent or cure COVID-19 viral infection, herbs and spices may constitute excellent bioresourses from which active molecules can be obtained.

Nigeria is a country which has recorded a great number of recoveries from COVID-19 infections. While vaccine

CONTACT Olanipekun Eunice Bolatito pekuntito@gmail.com Department of Chemical, Geological, and Physical Sciences, Kwara State University, Malete, Nigeria.
This article has been republished with minor changes. These changes do not impact the academic content of the article.
© 2022 Informa UK Limited, trading as Taylor & Francis Group

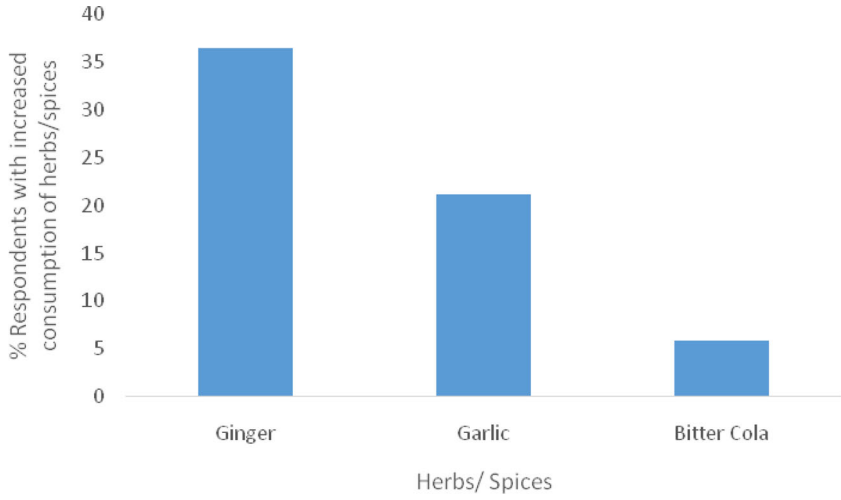


Figure 1. Number of respondents who increased their consumption of certain herbs and spices after the declaration of the COVID-19 pandemic.

Table 1. Docking scores of promising phytochemical ligands and control with SARS-CoV-2 target protein (3-chymotrypsin-like protease).

Herb/Spice	Compound	CID	Docking Scores (7C6U)	Docking Scores (7B3C)
Ginger	6-gingerol	442793	−4.11	−2.764
	8-gingerol	168114	−4.858	−3.174
	10-gingerol	168115	−4.924	−3.33
	6-shogaol	5281794	−2.595	−2.866
	8-shogaol	6442560	−4.365	−2.525
	10-shogaol	6442612	−3.979	−3.925
	6-gingerdione	162952	3.225	−3.374
	8-gingerdione	14440537	−4.24	−4.562
	10-gingerdione	14440539	−5.532	−4.225
	Zingerol	3016110	−5.033	−3.685
	6-Dehydrogingerdione	22321203	−3.78	−3.165
Garlic	Allicin	65036	−3.569	−1.752
	Diallylsulphide	16590	−1.872	–
	(E) Ajoene	5386591	−1.93	0.494
	Diallyltrisulphide	16315	0.862	–
	S-allylcystein	9793905	−0.683	−2.483
	Alliin	121922	−3.231	3.229
	2-vinyl-4H-1,3-dithiin	133337	−5.655	−4.205
	(Z) Ajoene	9881148	−1.723	0.293
	Allyl methyl sulfide (AMS)	66282	−2.977	–
Bitter Cola	Kolanone	11953940	−1.355	−2.403
	Amentoflavone	5281600	−6.936	−5.861
	24-methylenecyclartenol	94204	−3.959	−2.775
	Cyclartenol	12760132	−3.007	−2.649
	Garcinia biflavonoid I	161087	−7.489	−5.995
	Garcinia biflavonoid II	161259	−7.626	−6.019
	Kolaflavonone	155169	−6.562	−5.924
Control	Hydroxychloroquine	3652	−5.362	−3.956
	Remdesivir	121304016	−7.292	−5.753

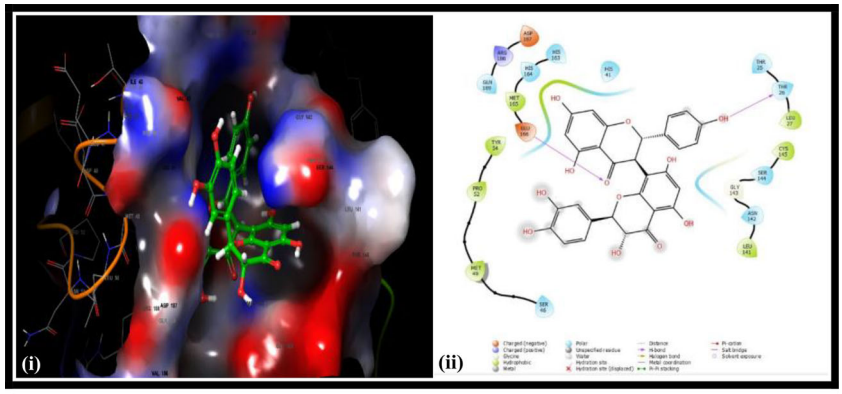
development and distribution continues, it may be wise to focus on prefered herbs/spices which are commonly consumed in Nigeria and identify the inherent phytochemicals which may be useful in anti-COVID-19 drug development. Although Sen et al. (2020) recently identified SARS-COV-2 inhibitors from 10 important spices, the methodology of this research is different and bitter cola, an important herb which is herein investigated was not included in their report.

The aim of this research, therefore, was to use *in silico* tools (molecular docking, pharmacophore modelling and molecular dynamics simulation) to identify known phytochemicals in commonly consumed herbs/spices which can effectively act as inhibitors of 3-chymotrypsin-like protease (3CLPro) and RNA dependent – RNA polymerase (RdRp) of SARS-CoV-2.

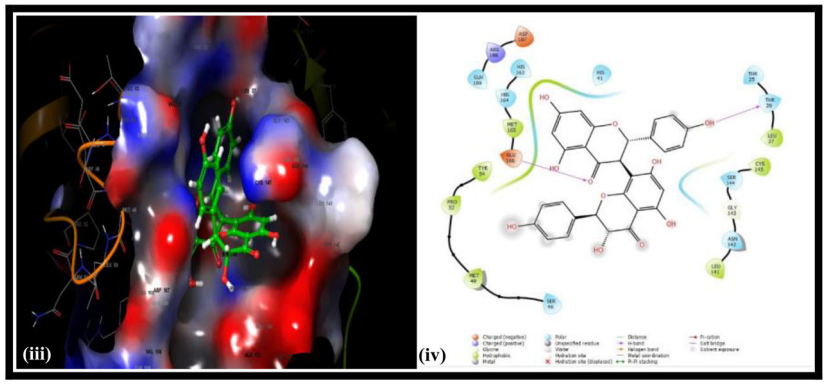
2. Materials and methods

2.1. Selection of ligands

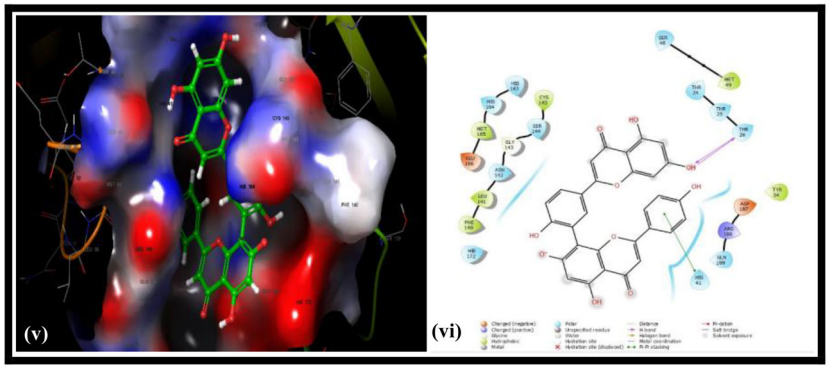
A google questionnaire was generated to study the dietary behavior of a section of Nigerians after the emergence of the COVID-19 pandemic. The data was collected between August and November 2020 from 91 respondents.



(i, ii) Garcinia biflavonoid II



(iii, iv) Garcinia biflavonoid I



(v, vi) Amentoflavone

Figure 2. The interaction of top-ranking phytochemicals with 3CL-pro: Left 3 D; Right 2 D.

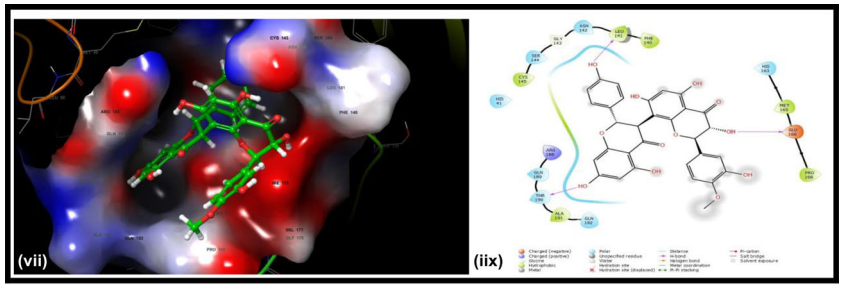
Particularly, each respondent was to tell if he took more of the common Nigerian spices, herbs, fruits or vitamin C after the pandemic began. The respondents also stated if they took ill within the period under study. Three herbs/spices with the highest number of usages were subjected to literature search in order to reveal known phytochemicals which have earlier been isolated from or identified in them. The search topic for each herb in science direct search engine was: Main/Active constituents of Ginger, Garlic and bitter cola. The focus of the search was on review articles/chapters in books with no restriction on the year of publication. The 3-D structures of the phytochemicals were then retrieved from the PubChem server and were used as ligands in the molecular docking experiment.

2.2. Ligand preparation

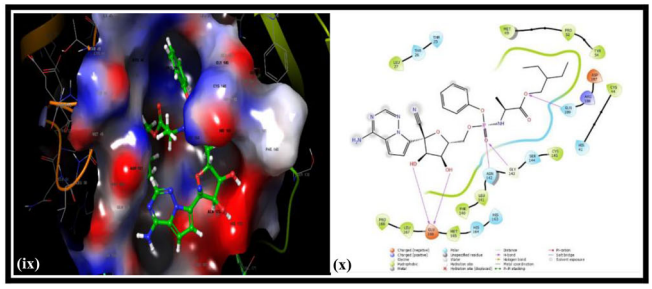
Ligprep module (Schrödinger Suite 2020-3) was used to prepare the twenty-seven (27) compounds from ginger, garlic and bitter cola for molecular docking. At a physiological pH of 7.2 ± 0.2, the possible ionization states for each ligand structure (low-energy 3 D: correct chirality) were generated. The specific chirality of each ligand was retained while computing their stereoisomers.

2.3. Protein preparation

The crystal structure of two drug targets of SARS-COV-2: SARS-CoV-2 Main Protease (PDB ID: 7C6U) and SARS-CoV-2



(vii, iix) Kolaflavone



(ix, x) Remdesivir

Figure 2. (Continued)

RNA-dependent RNA polymerase (PDB ID: 7B3C) were retrieved from Protein Data Bank (PDB) repository. The protein wizard panel of Glide (Schrödinger Suite 2020-3) was used to carry out the protein preparation where, there was addition of hydrogen, assignment of bond orders and creation of disulfide bonds. Also, missing loops and side chains were filled using prime. Water molecules beyond 3.0 Å of the heteroatoms were removed and the structure was minimized using OPLS2005 and optimized using PROPKA (Olsson et al., 2011). Subsequently, the receptor grid file was generated to define the binding pocket for the ligands.

2.4. Receptor grid generation

This is the process that defines the size as well as position of the protein's active site for ligand docking. The receptor grid generation tool of Schrödinger Maestro 12.5 was used to define the scoring grid based on the co-crystalized ligand (SARS-CoV-2 complexed with GC376 and SARS-CoV-2 RNA-dependent RNA polymerase with Remdesivir). Using the scale of 1.0 and partial cut off of 0.25, the nonpolar receptor van der Waals (vdW) radius scaling factor was determined.

2.5. Protein-ligand docking

The molecular docking studies was carried out by generating receptor grid file using the Glide tool of Schrödinger Maestro 12.5. Standard precision (SP), flexible ligand sampling, and extra precition (XP) (ligand sampling set to none – refine only) were the docking parameter used in the docking process. 0.15 partial charge cut-off for ligand atoms and 0.80 vdW radius scaling factor was used (Johnson et al., 2021).

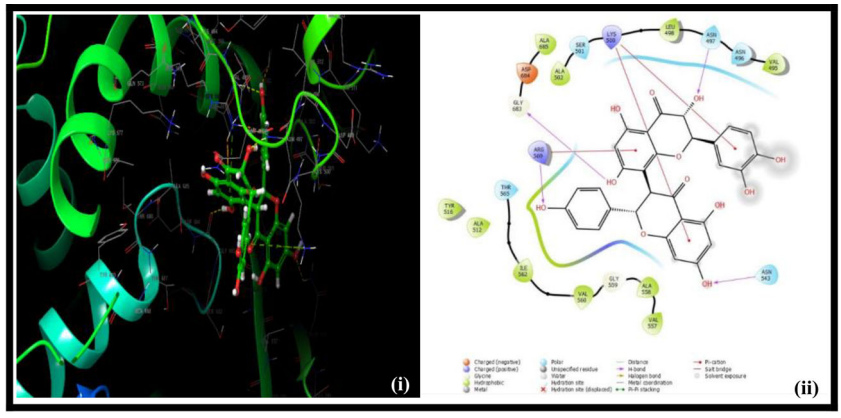
2.6. Receptor-ligand complex pharmacophore modelling

The first four compounds ranked with highest binding affinity against the target protein was used to develop a receptor-ligand complex pharmacophore model using PHASE.

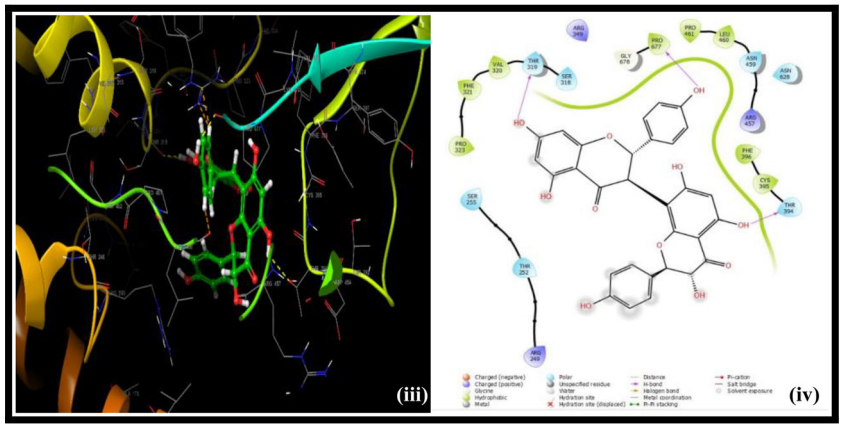
Auto (E-pharmacophore) method was employed with the hypothesis was set at maximum number of features to be generated as 7, minimum feature-feature distance as 2.00, minimum feature-feature distance for the same type of feature at 4.00 and vectors as donors.

2.7. Molecular dynamics simulation

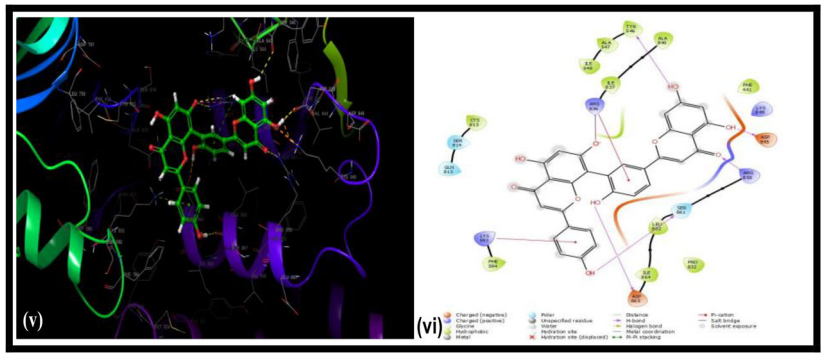
After the molecular docking studies, Desmond, a package from Schrödinger LLC, was used to carry out molecular dynamics simulation on the protein-ligand complexes for 100 nanoseconds. By observing static views of molecular docking results, one could capture the pose of ligands in the protein binding pocket. In brief, Protein Preparation Wizard tool of Maestro was used to preprocess the protein–ligand complex, which included complex optimization and minimization. Default parameters were retained for these processes. The 'System Builder' tool of maestro was used to prepare all of the systems. TIP3P (Transferable Intermolecular Interaction Potential 3 Points) was chosen as a solvent model with an orthorhombic box of 10x10x10 Å. Counter ions (Na^+ or Cl^-) were added to the models to make the system electrically neutral. In the simulation of the Protein-ligand complexes, the OPLS_2005 force field parameters were used. Before the simulation, the models were relaxed. For all the MD simulation, the *NPT* ensemble with 300 K temperature and 1 atm pressure was applied. Salt (0.15 M NaCl) was added to mimic physiological ambience. MD simulations were run on a Dell Precision T7810 Workstation with an Nvidia GeForce RTX 3070 8GB graphics



(i, ii) Garcinia Biflavonoid 2



(iii, iv) Garcinia Biflavonoid 1



(v, vi) Amentoflavone

Figure 3. The interaction of top-ranking phytochemicals with RdRp: Left 3 D; Right 2 D.

processing unit (GPU). After every 100 ps, the trajectories were saved for analysis, and the simulation's stability was determined by measuring the root mean square deviation (RMSD) of the protein and ligand over time.

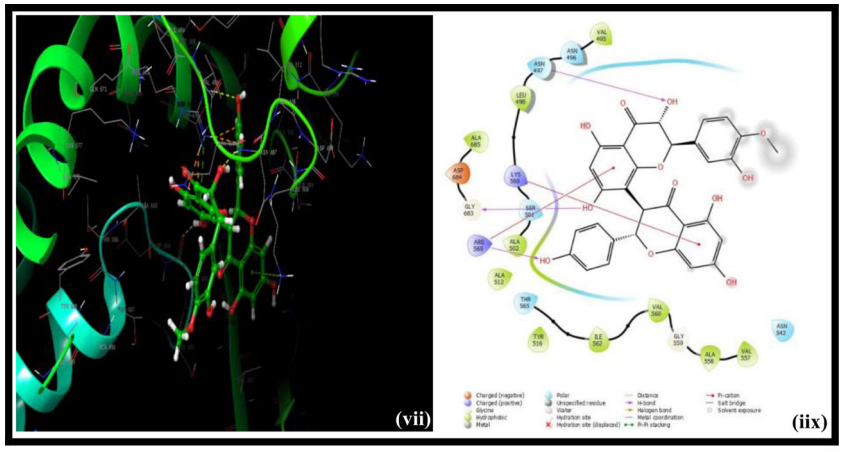
2.8. ADMET and drug-likeness prediction

ADMET is an acronym that describes the absorption, distribution, metabolism, excretion, and toxicity of molecules in the human body. SwissADME and admetSAR web servers were used for this prediction. Among other factors, bioavailability, solubility, lipophilicity, and druglikeness were predicted for the top-ranking phytochemicals with promising docking scores.

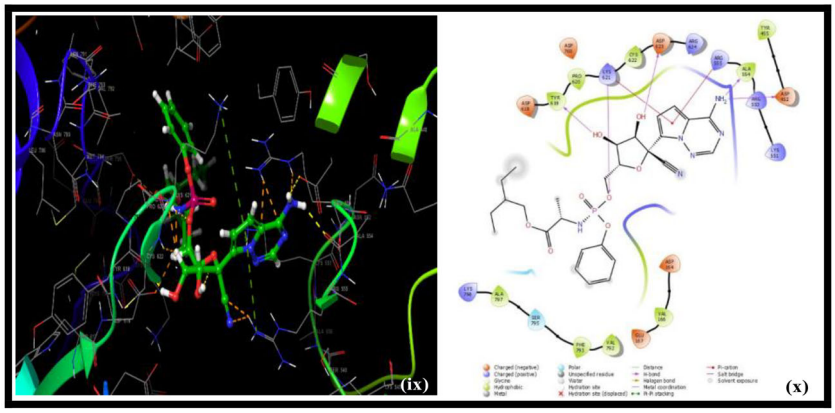
3. Results and discussion

3.1. Selection of phytochemical ligands

The phytochemical ligands employed in this study were selected based on the data from a survey (google questionnaire) in which each of ninety-one (91) respondent stated the herbs/spices which he/she consumed more since the advent of the COVID-19 pandemic. 37.4% of the respondents



(vii, iix) Kolaflavonone



(ix, x) Remdesivir

Figure 3. (Continued)

resided in Kwara State (North Central Nigeria) while 15.4, 9.9, 9.9, 5.5, 3.3, 3.3 and 2.2% resided in Oyo, Lagos, Abuja, Ogun, Kogi, Kaduna and Abia States respectively. 1.1% of the respondents resided in each of the following states: Delta, Ekiti, Osun, Benue, Plateau, Taraba, Rivers, Kano, Ondo, Edo.

The responses showing the number of mentions for each herb/spice are as shown in Figure 1.

Out of 85 respondents, 36.5%, 21.2%, 5.9%, respectively took more of ginger, garlic, and bitter cola. Consequently, 27 phytochemicals which have been earlier identified in or isolated from Ginger, garlic and bitter cola, selected from literature (Iwu et al., 2002; Mahomoodally et al., 2021; Mikaili et al., 2013). The PubChem ID of each molecule was used to retrieve its structure from PubChem server before docking. Other herbs/spices which were listed, but not considered for further study (because of lower frequency of mention) included turmeric, black pepper, pepper, lemon and bitter leaf.

3.2. Molecular docking

In molecular docking, the docking score is a major parameter which indicates the strength of interaction between the ligand and the receptor. The lower the docking score, the stronger the interaction. Among the phytochemicals and the control drugs (hydroxychloroquine/remdesivir) that were subjected to the molecular modelling with the two SARS-CoV-2 target protein receptors, Garcinia biflavovoid I, Garcinia biflavovoid II, amentoflavone and kolaflavonone displayed impressive docking scores as shown in Table 1.

The 3CL-pro/Garcinia biflavonoid II complex which shows 2 hydrogen bond interactions (with THR 26 and GLU 166) had the lowest docking score (-7.626). The higher docking score that was observed in Garcinia biflavonoid I shows that the extra OH functional group in Garcinia biflavonoid II was responsible for the observed variation. Only one hydrogen bond was observed along with some pi-pi stacking in the 3CL-pro/amentoflavone complex and thus, the higher docking score. The docking score in the 3CL-pro/remdesivir complex (-7.292), despite the 4-hydrogen bonding (GLY 189, GLY 143 and GLU 166), was slightly higher than that of Garcinia biflavonoid II. Similarly, the docking score of kolaflavonone with three hydrogen bond interactions (LEU 141, THR 190 and GLU 166) was higher (-6.562). This suggests that the hydrogen bond interaction with THR:26 of 3CLPro is of immense importance in the 3CLPro drug-ligand interaction.

A close observation of the RdRp-phytochemical interaction showed less impressive docking scores compared to the 3CLPro drug target. Worthy of note is the fact that Garcinia biflavonoid II also exhibited the lowest docking score (-6.019) and closely followed by Garcinia biflavonoid I,

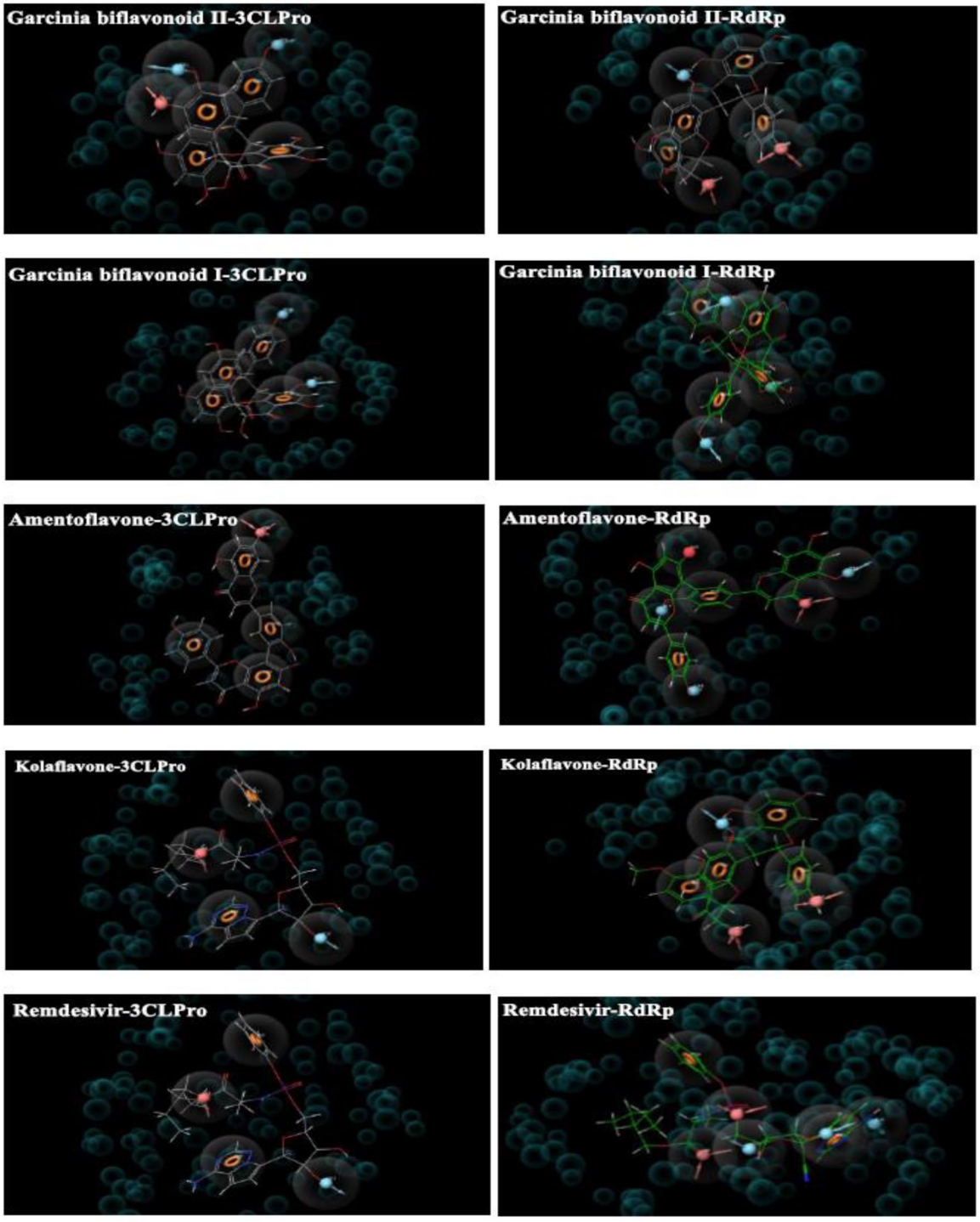


Figure 4. Pharmacophore models of garcinia biflavonoid I, garcinia biflavonoid II, amentoflavone, kolaflavone and remdesivir on 3-chymotrypsin-like protease (3CLPro) and RNA dependent – RNA polymerase (RdRp) of SARS-CoV-2. R = Aromatic ring. D = Hydrogen bond Donor. A = Hydrogen bond acceptor.

Kolaflavonone and amentoflavone. The 3 D and 2 D pictures of these drug target-phytochemical complexes are as shown in Figures 2 and 3.

The results obtained in this study agrees with the findings of Swargiary et al. (2020) where amentoflavone (along with gallocatechin gallate) was reported as the most promising potential inhibitors of 3CL-pro and Papain-like proteins among 32 anti-protease phytochemicals. In fact, flavonoids have been generally found to possess antiviral properties against SARS-and MERS-CoV via the inhibition of 3CLpro or PLpro (Solnier & Fladerer, 2020). Hence, our study supports the application of flavonoids as a complementary approach in COVID-19 therapy. The fact that the phytochemicals with the lowest (best) docking scores (garcinia biflavonoid II, garcinia biflavonoid I, amentoflavone and kolaflavanone) are all constituents of bitter kola is worthy of note. Bitter kola has been earlier reported for its an antibacterial, antiviral and anticancer properties (Icheku et al., 2018).

Hence, further studies on the phytochemical constituents of bitter kola may lead to the discovery of safe and effective

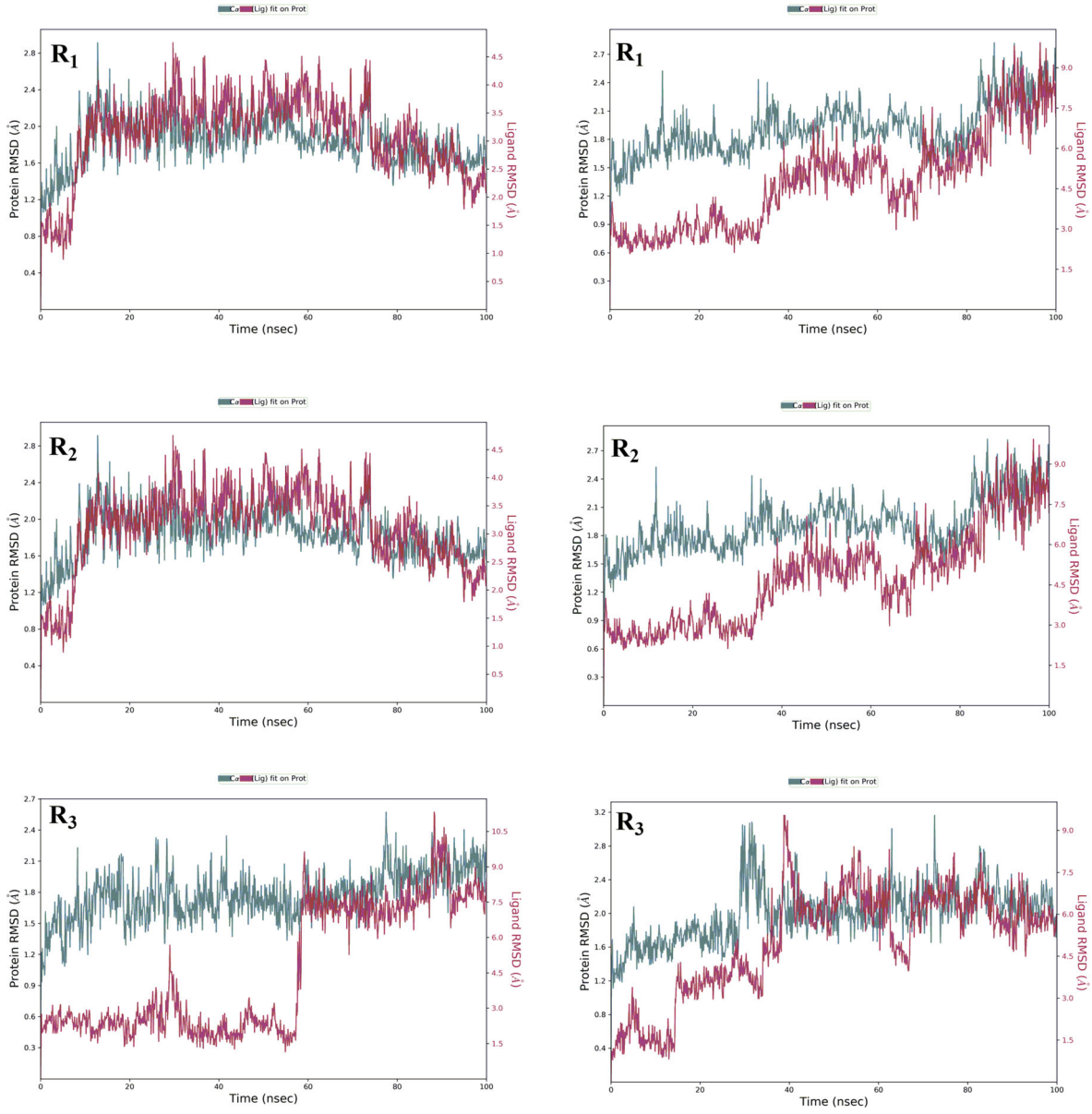


Figure 5. Protein and protein – ligand RMSD of the Cα atoms of 3CLpro protein and garcinia biflavonoid I (left) garciniabiflavonoid II (right) with time. The left y-axis shows the variation in the 3CLpro RMSD while the right y-axis shows the RMSD variation of the complexes with time.

pharmaceuticals for the treatment of SARS-CoV-2 disease. Although the constituents of bitter kola showed more promise as inhibitors of SARS-CoV-2 proteins, the constituents of ginger may offer their health defense via another mechanism. It is important to note that ginger is rich in zinc and zinc containing compounds have been established as antiviral agent (Attia et al., 2021; Banihani, 2018).

3.3. The pharmacophore modelling

Figure 4 shows the pharmacophore models of the compounds and Remdesivir on 3-chymotrypsin-like protease (3CLPro) and RNA dependent – RNA polymerase (RdRp) of SARS-CoV-2. The model of Garcinia biflavonoid I, Garcinia biflavonoid II, kolaflavone and amentoflavone revealed that their aromatic rings contributed to their high binding affinity for the protein target. In addition to this, the Garcinia biflavonoid I model showed that two of its phenolic -OH groups act as hydrogen bond donor to 3CLPro. The model for Garcinia biflavonoid II is similar to that of Garcinia biflavonoid I except for the positions of the two hydrogen bond donors and the additional hydrogen bond acceptor (A11) on R24. However, amentoflavone possess only one hydrogen bond acceptor (A9) on the aromatic ring (R20) while kolaflavone might have interacted with the protein target via other mechanisms. Remdesivir on the other hand displayed a hydrogen bond donor, a hydrogen bond acceptor and two aromatic ring interactions. Therefore, the higher binding affinity of Garcinia biflavonoid II compared to the standard drug remdesivir could be attributed to the presence of higher number of hydrogen bond donors and higher aromatic interactions between the compound and the protein.

Furthermore, the pharmacophore models of the phytochemicals (Garcinia biflavonoid I, Garcinia biflavonoid II, amentoflavone, kolaflavone) and Remdesivir on RdRp

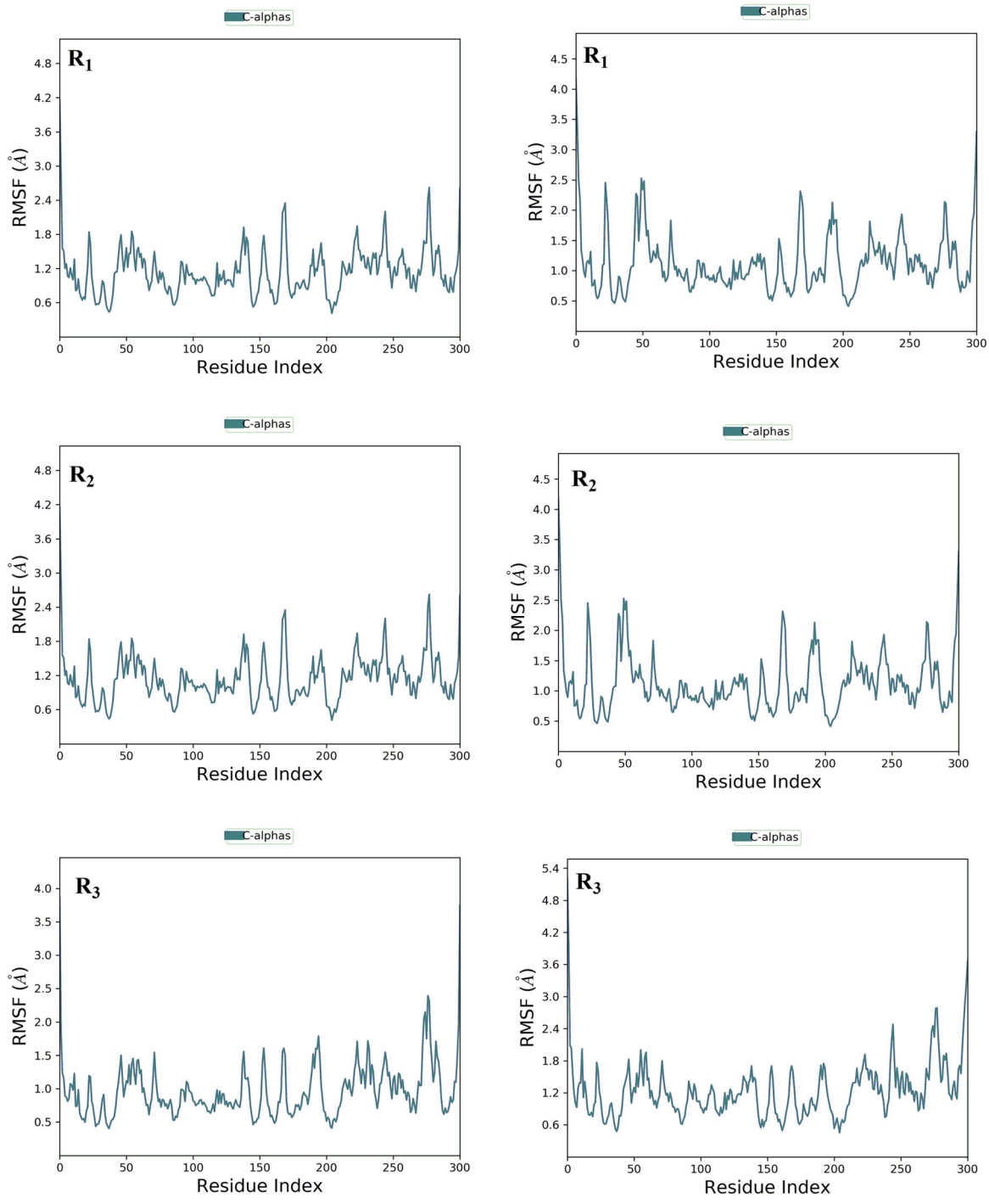


Figure 6 **a.** Protein root mean square flunctuations of Garcinia biflavonoid I (left) and Garcinia biflavonoid II (right), with 3CLPro.

revealed that; in addition to the presence of hydrogen bond donors and acceptors, all the phytochemicals possessed higher aromatic interactions with the protein than the standard drug. Hence, they displayed higher docking scores compare to the standard drug. These phytochemicals can therefore be developed into relevant anti- COVID-19 drugs (Figure 4).

3.4. Molecular dynamics simulation

MD simulations tend to compute atom movements over time by integrating Newton's classical equation of motion. The ligand binding status in the physiological milieu was predicted using simulations. The MD simulation of 7C6U-GB I and 7C6U-GB II complexes as well as that of the unligated 7C6U were performed for 100 ns. The simulation was repeated trice (n = 3) to establish reproducibility of the results. Root Mean Square Deviation (RMSD) and Root Mean Square Fluctuation (RMSF) of the MD trajectories were initially used to comprehend the stability and possible fluctuations in the complexes. Afterwards, the dG bind free energy of the complexes were calculated using MM-GBSA analysis. The RMSD values of 3CLPro (unligated) protein rose from 0 to 10 ns while that of 3CLPro -

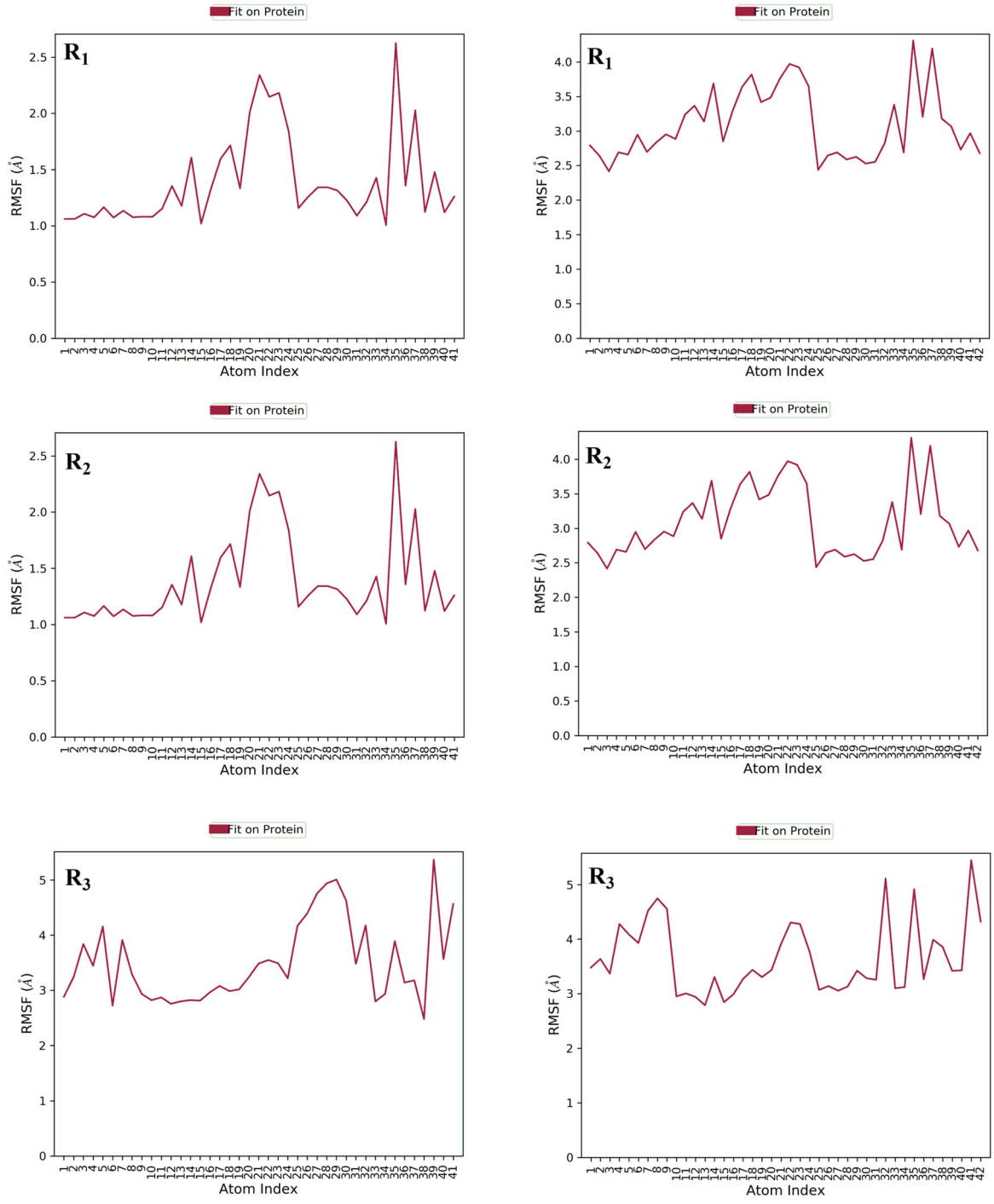


Figure 6 **b.** Ligand root mean square flunctuations of Garcinia biflavonoid I (left) and Garcinia biflavonoid II (right), with 3CLPro.

Garcinia Biflavonoid I complex remained constant (~0.8 Å) from 0 – 6 ns (replicates 1 and 2). Afterwards, both RMSDs became equilibrated at 10 ns. However, the RMSD values of both 3CLPro (unligated) and 3CLPro - Garcinia Biflavonoid II complex increased from 0 to 30 ns before converging and equilibrating at 40 ns. Although with slight deviations, they both remained stable until the end of the simulation time. These results preliminarily show that there was no major deviation or conformation adjustment as the interaction of these ligands with the protein is stable (Figure 5).

The RMSF is used for quantifying local changes/amino acid fluctuations along the protein chain (Islam et al., 2021). The RMSF of alpha carbons shows that Garcinia biflavonoid II exhibited more fluctuations as shown in Figure 6 (Replicates 1, 2 and 3).

Less RMSF fluctuation indicates more interaction with the active site (Jakhmola et al., 2021). Hence, the average RMSF values of Garcinia biflavonoid 1 and Garcinia biflavonoid 2 as shown in Figure 6 reveals that Garcinia Biflavonoid I exhibits less conformational change than Garcinia biflavonoid II, even though GB2 had a better docking score.

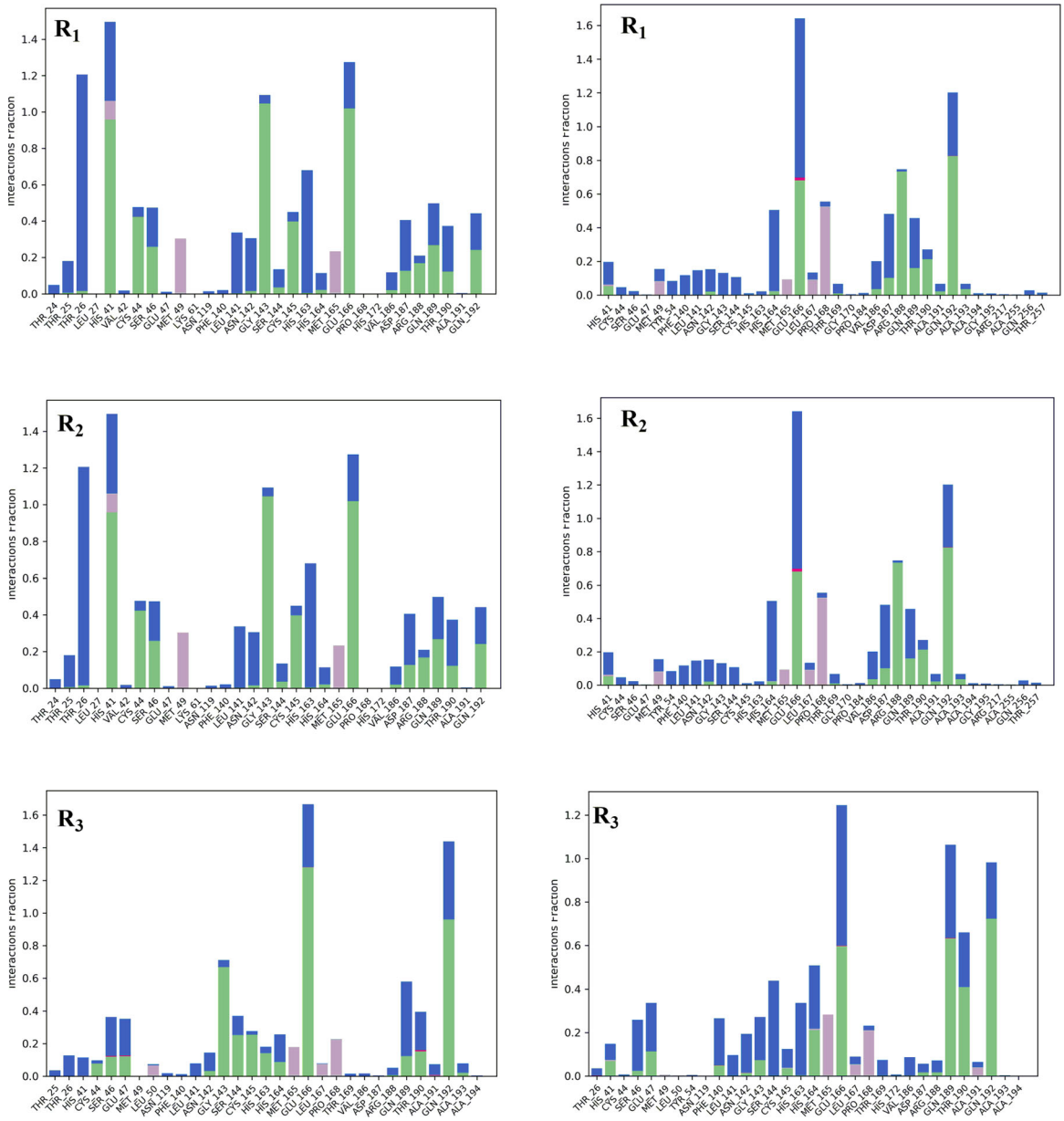


Figure 7. Protein-Ligand contact histogram 3CLPro- Garcinia biflavonoid I (left), 3CLPro- Garcinia biflavonoid II (right).

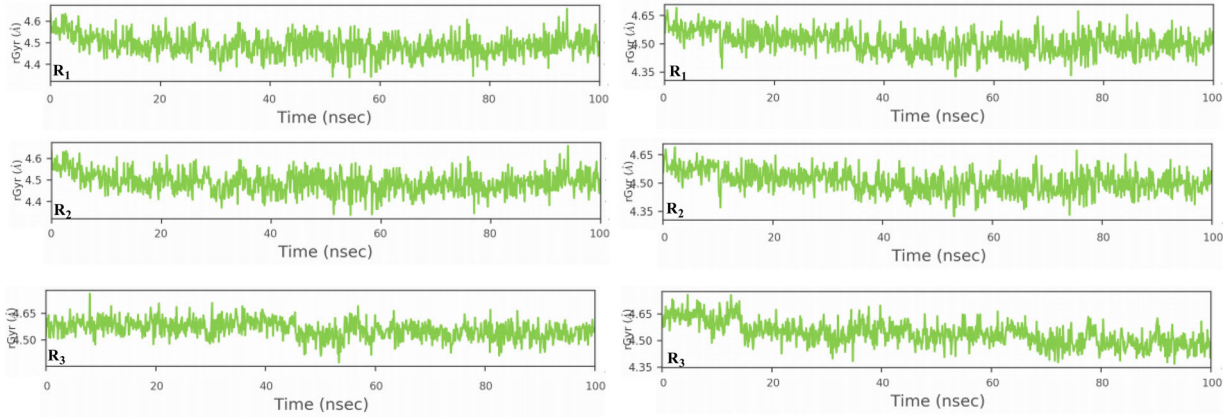


Figure 8. Radius of gyration of 3CLPro- Garcinia biflavonoid I (left), 3CLPro- Garcinia biflavonoid II (right).

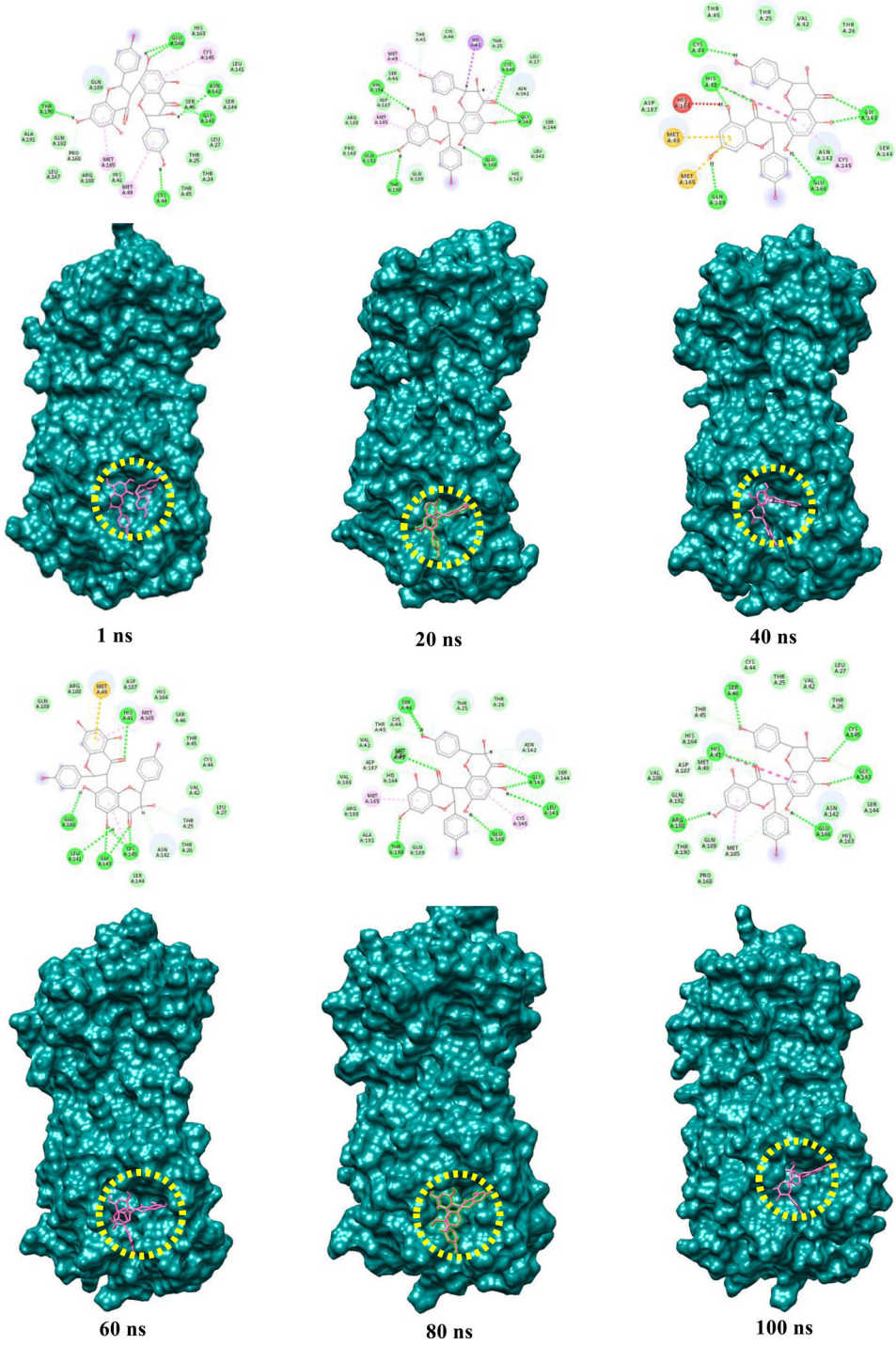


Figure 9 **a**. Binding interactions 3CLpro protein and Garcinia biflavonoid I complex at different time intervals of MD simulations.

Furthermore, Amino acid residues: HIS41, GLY143 and GLU166 were the most prominent points of ligand-protein interaction in 3CLPro-Garcinia biflavonoid I complex while GLN192, ARG188, GLU166 amino acid residues were points of high protein-ligand interaction in 3CLPro- Garcinia biflavonoid II (Figure 7) (Replicates 1 and 2). Although a slight variation was observed in replicate 3, GLU166 remained a most prominent point of protein-ligand contact for the two complexes throughout the simulation time.

The determination of Radius of gyration (Rg) and Solvent Assessible Surface Area (SASA) from the MD tragectories also assisted in the post MD analysis (Figure 8). While Rg shows compactness in the systems, SASA usually aids in assessing the degree of expansion of protein volume in each system (Swargiary et al., 2020).

The 3CLPro-GB2 complex had higher radius of gyration throughout the simulation period. This implies that the 3CLPro-GB2 complex had more flexibility/lower rigidity, compared to the 3CLPro-GB1 complex.

In order to further understand the nature of the protein-ligand complexes, the binding stability of the complexes involving the highest ranking phytochemicals was observed

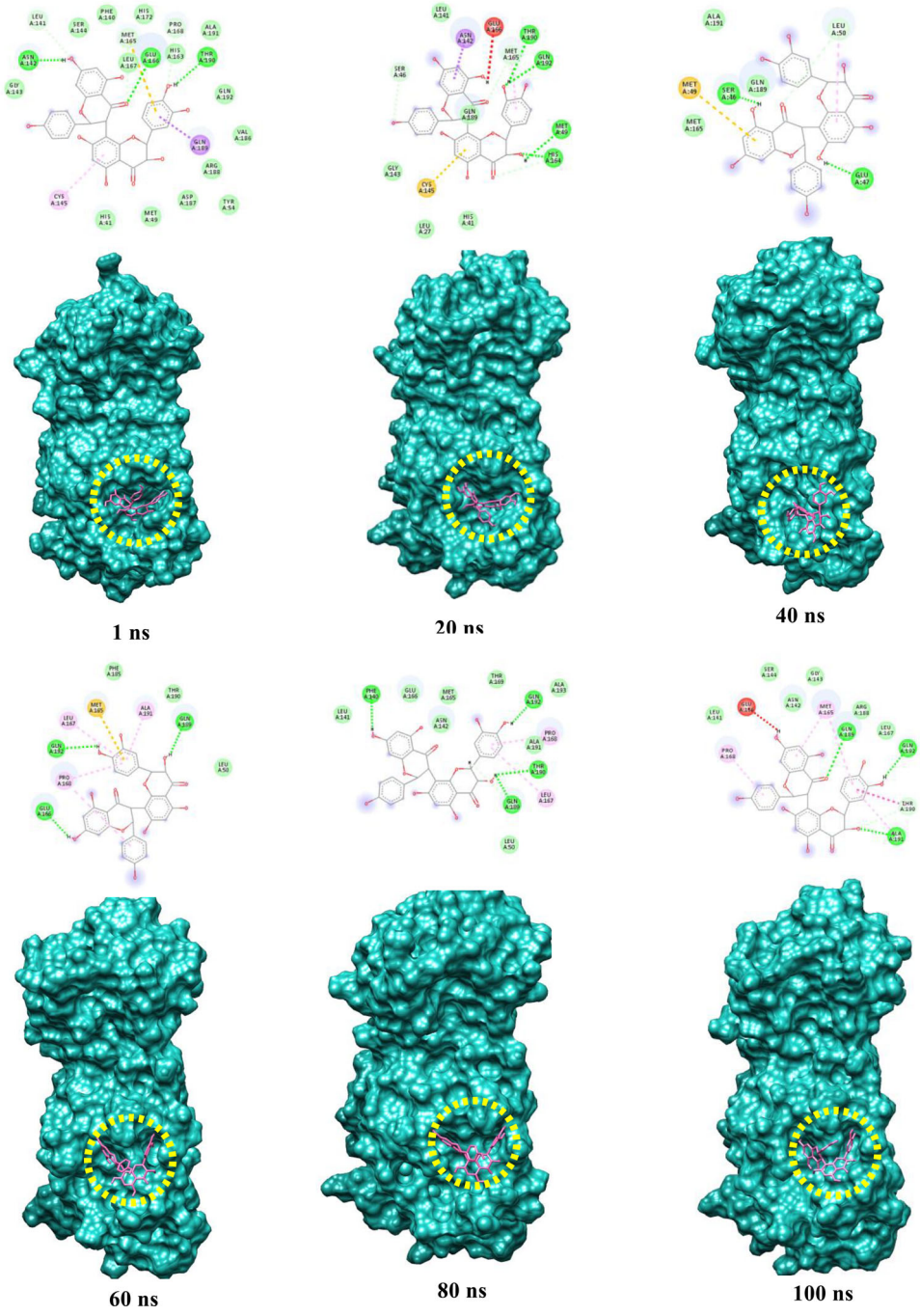


Figure 9 **b**. Binding interactions 3CLpro protein and Garcinia biflavonoid II complex at different time intervals of MD simulations.

Table 2. 3CLpro-Garcinia biflavonoid I and 3CLpro-Garcinia biflavonoid II interactions at different MD simulation time.

	3CLpro-Garcinia biflavonoid I Interactions						
	Van der Waals	Conventional Hydrogen Bond	Unfavorable Donor-Donor	Pi- Sulfur	Pi-Alkyl	Pi- Sigma	Pi-Pi stacked
1 ns	14	6	–	–	3	–	–
20 ns	11	7	–	–	2	1	–
40 ns	7	7	1	2	1	–	1
60 ns	11	7	–	1	1	–	–
80 ns	10	8	–	–	2	–	–
100 ns	15	6	–	–	1	–	1
	3CLpro-Garcinia biflavonoid II Interactions						Amide Pi Stacked
1 ns	14	3	–	1	1	1	–
20 ns	5	4	1	1	1	1	–
40 ns	3	2	–	1		–	–
60 ns	3	3	–	1	4	–	–
80 ns	8	4	–	–	2	–	–
100 ns	6	3	1	–	4	–	1

with time (Figure 9 and Table 2). The 3CLPro – Garcinia biflavonoid I complex had 6, 7, 7, 7, 8, 6 hydrogen bond interaction for 1, 20, 40, 60, 80 and 100 ns respectively while the 3CLPro – Garcinia biflavonoid II complex possessed 3, 4, 2, 3, 4, 3 for 1, 20, 40, 60, 80 and 100 ns respectively. At 100 ns, six amino acid residues (ARG188, HIS41, SER46, CYS145, GLY143, GLU 166) in 3CLPro interacted with Garcinia biflavonoid I via hydrogen bond while only 3 amino acid residues (GLN189, GLN192, ALA191) interacted with Garcinia biflavonoid II. GLU166 has earlier been reported as a crucial amino acid residue in 3CLPro (Swargiary et al., 2020). Similarly, the molecular docking results and the molecular dynamics simulation (at 1 ns) shows that GLU 166 formed a hydrogen bond with the ligand - Garcinia biflavonoid II. However, the interaction was not sustained throughout the 100 ns simulation. This must have contributed to the binding energy that was observed in the 3CLPro-Garcinia biflavonoid II.

The Binding free energy of the two complexes were calculated, using the Molecular Mechanics – Generalized Born Surface Area (MM-GBSA) analysis. This method, which has been adjudged to be more reliable than glide scores, has different energy components such as ΔG_{bind} coulomb, ΔG_{Bind} vdW and ΔG_{bind} solv GB (Forouzesh & Mishra, 2021). A thousand snapshots were taken from the MD simulation and the average binding free energy of each replicate are as shown in Table 3. The replicates were analyzed using one-way anova (with tukey posthoc comparisons). The mean values of the ΔG_{Bind} from the replicates were compared using one-way anova and no significant difference was observed between the average ΔG_{Bind} values ($P > 0.05$).

A significant observation in this study is the fact that the compound with the second highest molecular docking score (Garcinia biflavonoid I), displayed less comformational changes/more stability with 3CLPro compared with the compound that had the highest molecular docking score (as revealed by molecular dynamics simulation). The MM-GBSA values also corroborates this finding as Garcinia biflavonoid 1 had an impressive ΔG_{bind} of −97.9262 kcal/mol. Hence, the results presented herein are worthy of further research attention.

Table 3. Binding free energies of 3CLPro-Garcinia Biflavonoid I and 3CLPro-Garcinia Biflavonoid II complexes.

	ΔG_{Bind} (kJ/mol)	
Replicate	Garcinia biflavonoid I	Garcinia biflavonoid II
1	−97.9262 (10.0019)	−57.0351 (10.6855)
2	−97.9262 (9.9969)	−57.0351 (10.6801)
3	−73.4162 (12.1308)	−56.8948 (11.5184)

(): Standard deviations.

3.5. ADMET prediction and drug-likeness properties

The hydrophilicity of the phytochemicals is usually expressed by the log P value which is calculated from the n-octanol/water partition. The log P values for the phytochemicals in Table 4 are less than 5, thus indicating a druglikeness of the compounds (Gao et al., 2017)

Since higher log P values indicate poor absorption and permeation (Gao et al., 2017), garcinia biflavonoid I is expected to exhibit the most drug-like (lowest) log P value. Worthy of note as well, is the fact that the Log P value of amentoflavone was slightly lower than that of remdesivir. All the phytochemicals had poor solubility predictions. Similarly, all the phytochemicals had low gastrointestinal absorption predictions while the molecular weight of amentoflavone (538.46) places it ahead of remdesivir (602.58) in the drug-like prediction. Lower TPSA has been reported to be favourable for drug-like properties (Nisha et al., 2016). Hence, membrane permeability is predicted to be more diminished in remdesivir than in amentoflavone. Other drug-like prediction details are contained in Table 4.

Based on the predicted toxicity profile of the phytochemicals in Table 5, only Garcinia Biflavonoid II is mutagenic while the phytochemicals either belong in acute oral toxicity class II or III. Mostly, class II acute oral toxicity is expected to have moderate toxicity while those in class III are slightly toxic. In this case, Remdesivir and Kolaflavone appear to be slightly toxic while, amentoflavone and others are predicted to be moderately toxic. In addition, all the phytochemicals may have potential to cause liver injury as well as fish aquatic toxicity. In contrast, only Remdesivir hold toxic potential against crustacea and it's able to cross the blood brain barrier. Taken together, the *in*-silico toxicity profiling may indicate a need for caution in the repeated consumption of bitter kola as 75% of the phytochemicals investigated in this study, belong in toxicity class II. Only kolaflavone belongs in toxicity class III. Interestingly, the majority of investigations

Table 5. Toxicity profile of promising phytochemical ligands.

Molecules	Amento	GB2	Kolaflav	GB 1	Remdesivir
Ames mutagenesis	−	+	−	−	−
Acute Oral Toxicity (c)	II	II	III	II	III
Blood Brain Barrier	−	−	−	−	+
Carcinogenicity (binary)	−	−	−	−	−
Carcinogenicity (trinary)	NR	NR	NR	NR	NR
crustacea aquatic toxicity	−	−	−	−	+
Eye corrosion	−	−	−	−	−
Eye irritation	−	−	−	−	−
Fish aquatic toxicity	+	+	+	+	+
Honey bee toxicity	+	−	−	−	−
Hepatotoxicity	+	+	+	+	+
Acute Oral Toxicity	1.8223	2.7485	2.7422	2.4651	3.42793

Table 4. Drug-like properties of selected phytochemical ligands.

		Hydrogen bonds											
Molecules	MW	Donor	Acceptor	RB	iLogP	Lipinski #violation	MR	TPSA	Ali Class	GIA	BAS	PAINS #alerts	Brenk #alerts
GB2	574.49	8	12	3	1.98	3	144.37	214.44	P.S	Low	0.17	1	1
GB1	558.49	7	11	3	1.96	3	142.35	194.21	P.S	Low	0.17	0	0
Remdesivir	602.58	4	12	14	3.24	2	150.43	213.36	P.S	Low	0.17	0	1
Amentoflavone	538.46	6	10	3	3.06	2	146.97	181.8	P.S	Low	0.17	0	0

P.S = Poorly soluble, M.S = Moderately soluble, BAS = Bioavailability Score, GIA = Gastro Intestinal Absorption, RB = Rotatable Bonds, MW = Molecular Weight.

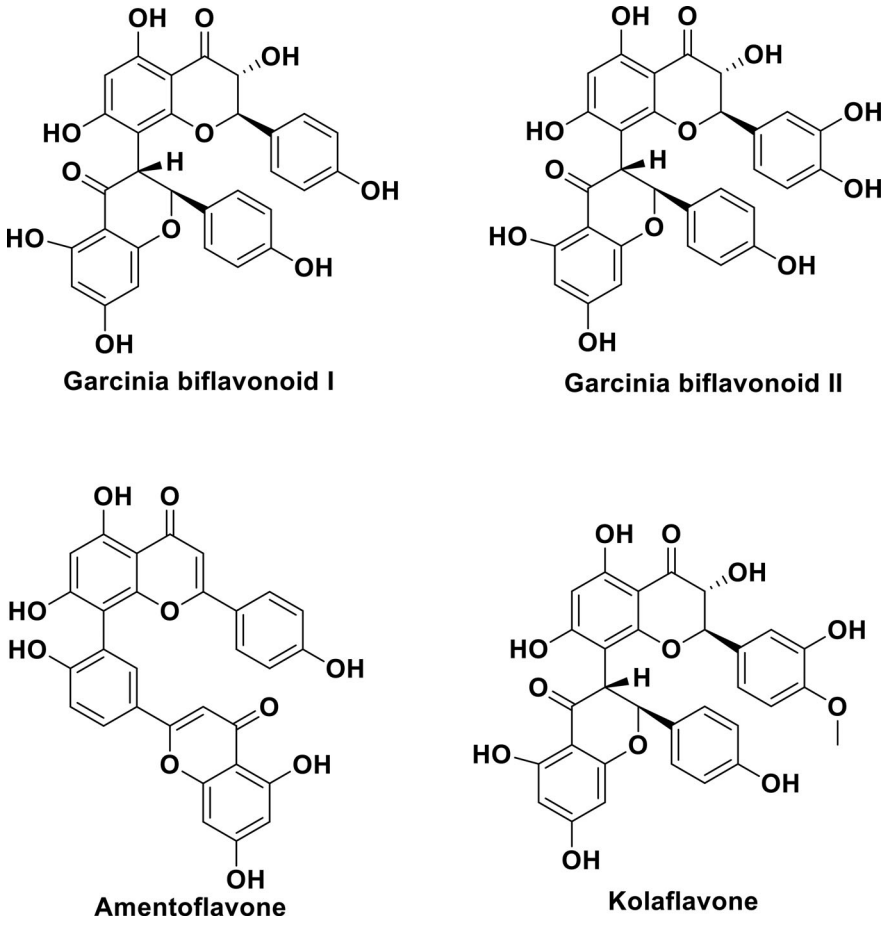


Figure 10. Molecular structure of the promising SARS-CoV-2 main protease and RNA dependent-RNA Polymerase inhibitors.

have been focused on the medicinal benefits of these natural products and their constituents. Meanwhile, little has been done to study the toxicity potential of these medicinal bioactives.

4. Conclusion

In conclusion, following an online survey of the consumption preference of herbs and spices, some phytochemicals of the commonly consumed herbs and spices in Nigeria were identified through a literature search. The identified phytochemicals were docked against 3CL-pro of SARS-COV-2 and four ligands namely Garcinia Biflavonoid I, Garcinia Biflavonoid II, amentoflavone, and kolaflavone (Figure 10) showed promising inhibitory potential. In the molecular dynamics simulation, Garcinia bioflavonoid I exhibited less conformational change and an impressive binding free energy with the SARS-CoV-2 main protease, 3CLPro. Hence, the compound could be further investigated for proper incorporation into the CoViD-19 therapeutic armory. However, a toxicity profiling revealed that these bioflavonoids are mostly moderately toxic, thereby necessitating caution in the indiscriminate and repeated consumption of bitter kola.

Disclosure statement

No potential conflict of interest was reported by the authors.

Funding

The author(s) reported there is no funding associated with the work featured in this article.

References

Adarkwah, M. A. (2021). "I'm not against online teaching, but what about us?": ICT in Ghana post Covid-19. *Education and Information Technologies*, *26*(2), 1665–1685. https://doi.org/10.1007/s10639-020-10331-z

Ang, L., Song, E., Lee, H. W., & Lee, M. S. (2020). Herbal medicine for the treatment of Coronavirus disease 2019 (COVID-19): A systematic review and meta-analysis of randomized controlled trials. *Journal of Clinical Medicine*, *9*(5), 1583. https://doi.org/10.3390/jcm9051583

Attia, G. H., Moemen, Y. S., Youns, M., Ibrahim, A. M., Abdou, R., & El Raey, M. A. (2021, January). Antiviral zinc oxide nanoparticles mediated by hesperidin and in silico comparison study between antiviral phenolics as anti-SARS-CoV-2. *Colloids and Surfaces. B, Biointerfaces*, *203*, 111724. https://doi.org/10.1016/j.colsurfb.2021.111724.

Banihani, S. A. (2018). Ginger and testosterone. *Biomolecules*, *8*(4), 119. https://doi.org/10.3390/biom8040119

Chakraborty, I., & Maity, P. (2020). COVID-19 outbreak: Migration, effects on society, global environment and prevention. *The Science of the Total Environment*, *728*, 138882 https://doi.org/10.1016/j.scitotenv.2020.138882

Dwivedi, Y. K., Hughes, D. L., Coombs, C., Constantiou, I., Duan, Y., Edwards, J. S., Gupta, B., Lal, B., Misra, S., Prashant, P., Raman, R., Rana, N. P., Sharma, S. K., & Upadhyay, N. (2020, July). Impact of COVID-19 pandemic on information management research and practice: Transforming education, work and life. *International Journal of*

Information Management, *55*, 102211. https://doi.org/10.1016/j.ijinfomgt.2020.102211

Forouzesh, N., & Mishra, N. (2021). An effective MM/GBSA protocol for absolute binding free energy calculations: A case study on SARS-CoV-2 spike protein and the human ACE2 receptor. *Molecules*, *26*(8), 2383. https://doi.org/10.3390/molecules26082383

Gao, Y., Gesenberg, C., & Zheng, W. (2017). Oral Formulations for preclinical studies: Principle, design, and development considerations. In Yihong Qiu, et al. (Eds.), *Developing solid oral dosage forms: Pharmaceutical theory and practice* (2nd ed., pp. 455–495). Academic press. https://doi.org/10.1016/B978-0-12-802447-8.00017-0

Icheku, V., Onianwah, I., & Nwulia, A. (2018). A descriptive cross-sectional study on various uses and outcomes of Garcinia kola among people of Oshimili North in the Delta State of Nigeria. *Ayu* , *39*(3), 132–138. https://doi.org/10.4103/ayu.AYU_195_16

Islam, R., Parves, M. R., Paul, A. S., Uddin, N., Rahman, M. S., Mamun, A. A., Hossain, M. N., Ali, M. A., & Halim, M. A. (2021). A molecular modeling approach to identify effective antiviral phytochemicals against the main protease of SARS-CoV-2. *Journal of Biomolecular Structure and Dynamics*, *39*(9), 3213–3224. https://doi.org/10.1080/07391102.2020.1761883

Iwu, M., Diop, A. D., Meserole, L., & Okunji, C. O. (2002). Garcinia kola: A new look at an old adaptogenic agent. In Iwu M. & Wootton, J. (Eds.), *Ethnomedicine and Drug Discovery*, Chapter 17 (p. 191). Amsterdam: Elsevir Science.

Jakhmola, S., Jonniya, N. A., Sk, M. F., Rani, A., Kar, P., & Jha, H. C. (2021). Identification of potential inhibitors against Epstein–Barr virus nuclear antigen 1 (EBNA1): An insight from docking and molecular dynamic simulations. *ACS Chemical Neuroscience*, *12*(16), 3060-3072. https://doi.org/10.1021/acschemneuro.1c00350

Johnson, T. O., Adegboyega, A. E., Iwaloye, O., Eseola, O. A., Plass, W., Afolabi, B., Rotimi, D., Ahmed, E. I., Albrakati, A., Batiha, G. E., & Adeyemi, O. S. (2021). Computational study of the therapeutic potentials of a new series of imidazole derivatives against SARS-CoV-2. *Journal of Pharmacological Sciences*, *147*(1), 62–71. https://doi.org/10.1016/j.jphs.2021.05.004

Johnson, T. O., Adegboyega, A. E., Ojo, O. A., Jega, A., Iwaloye, O., Ugwah-Oguejiofor, C., Asomadu, R., Chukwuma, I., Ejembi, S., & Ugwuja, E. (2021). Identification of possible inhibitors of SARS-CoV-2 main protease from some bioactive compounds of Artemisia Annua : An in silico approach. *Scientific Reports*, 1–15. https://doi.org/10.21203/rs.3.rs-612899/v1

López-Alcalde, J., Yan, Y., Witt, C. M., & Barth, J. (2020). Current State of Research about Chinese herbal medicines (CHM) for the treatment of coronavirus disease 2019 (COVID-19): A scoping review. *The Journal of Alternative and Complementary Medicine*, *26*(7), 557–570. https://doi.org/10.1089/acm.2020.0189

Mahomoodally, M. F., Aumeeruddy, M. Z., Rengasamy, K. R. R., Roshan, S., Hammad, S., Pandohee, J., Hu, X., & Zengin, G. (2021). Ginger and its active compounds in cancer therapy: From folk uses to nano-therapeutic applications. *Seminars in Cancer Biology*, *69*, 140–149. https://doi.org/10.1016/j.semcancer.2019.08.009

Mikaili, P., Maadirad, S., Moloudizargari, M., & Aghajanshakeri, S. (2013). Ijbms-16-1031- Garlic and Shallot. *Pdf. Iranian Journal of Basic Medical Sciences*, *16*, 1031–1048.

Nisha, C. M., Kumar, A., Nair, P., Gupta, N., Silakari, C., Tripathi, T., & Kumar, A. (2016). Molecular docking and in silico admet study reveals acylguanidine 7a as a potential inhibitor of β-Secretase. *Advances in Bioinformatics*, *2016*, 9258578 https://doi.org/10.1155/2016/9258578

Olsson, M. H. M., Søndergaard, C. R., Rostkowski, M. & Jensen, J. H. (2011). P ROP KA3: Consistent treatment of internal and surface residues in empirical p K a predictions. *J. Chem. Theory Comput*, *7*, 525–537.

Omonijo, D., O., Nnedum, O., A. U., Fadugba, A., O., Uche, O., C. O., & Makodi, B. N. (2013). Social vices associated with the use of Information Communication Technologies (ICTs) in a Private Christian Mission University, Southern Nigeria. *African Journal of Business Management*, *7*(31), 3078–3089. https://doi.org/10.5897/AJBM12.1193

Otu, A., Charles, C. H., & Yaya, S. (2020). Mental health and psychosocial well - being during the COVID - 19 pandemic : The invisible elephant in the room. *International Journal of Mental Health Systems*, *14*(1), 1–5. https://doi.org/10.1186/s13033-020-00371-w

Pramathesh, N. T., Sachi, S., Yadav, S., Khan, W., Afzal, M., Alari, A., Tahir, M., Hasnain, S., & Kumar, A. (2020, July). Global impacts of pre- and post-COVID-19 pandemic : Focus on socio-economic consequences. Sensors International*, 1*, 100042. https://doi.org/10.1016/j.sintl.2020.100042

Sahu, P. (2020). Closure of universities due to coronavirus disease 2019 (COVID-19): Impact on education and mental health of students and academic staff. *Cureus*, *2019*(4), 4–9. https://doi.org/10.7759/cureus.7541

Sen, D., Debnath, P., Debnath, B., Bhaumik, S., & Debnath, S. (2020, September). Identification of potential inhibitors of SARS-CoV-2 main protease and spike receptor from 10 important spices through structure-based virtual screening and molecular dynamic study. *Journal of Biomolecular Structure and Dynamics*, *18*, 1–22. https://doi.org/10.1080/07391102.2020.1819883

Solnier, J., & Fladerer, J. P. (2020). Flavonoids: A complementary approach to conventional therapy of COVID-19? *Phytochemistry Reviews*, *20*, 773-795. https://doi.org/10.1007/s11101-020-09720-6

Swargiary, A., Mahmud, S., & Saleh, M. A. (2020). Screening of phytochemicals as potent inhibitor of 3-chymotrypsin and papain-like proteases of SARS-CoV2: An in silico approach to combat COVID-19. *Journal of Biomolecular Structure and Dynamics*, 1–15. https://doi.org/10.1080/07391102.2020.1835729

Trinh, T. A., Park, J., Oh, J. H., Park, J. S., Lee, D., Kim, C. E., Choi, H. S., Kim, S. B., Hwang, G. S., Koo, B. A., & Kang, K. S. (2020). Effect of herbal formulation on immune response enhancement in raw 264.7 macrophages. *Biomolecules*, *10*(3), 424–416. https://doi.org/10.3390/biom10030424

Tripathi, D., Yi, G., & Vankayalapati, R. (2020). COVID-19: An update on vaccine development. *Indian J Rheumatol*, *15*, 70–72.

Wink, M., Ashour, M. L., & El-Readi, M. Z. (2012, April). Secondary metabolites from plants inhibiting ABC transporters and reversing resistance of cancer cells and microbes to cytotoxic and antimicrobial agents. *Frontiers in Microbiology*, *3*, 130–115. https://doi.org/10.3389/fmicb.2012.00130

World Health Organization. (2021). https://www.covid19.who.int; https://www.who.int/health-topics/coronavirus#tab=tab_1. accessed on 30th April, 2021

Zou, L., Dai, L., Zhang, X., Zhang, Z., & Zhang, Z. (2020). Hydroxychloroquine and chloroquine: A potential and controversial treatment for COVID-19. *Archives of Pharmacal Research*, *43*(8), 765–772. https://doi.org/10.1007/s12272-020-01258-7