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In-silico elucidation reveals potential phytochemicals against angiotensin-converting enzyme 2 (ACE-2) receptor to fight coronavirus disease 2019 (COVID-19)

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Abstract: The coronavirus (SARS-CoV-2) pandemic is rapidly advancing and spreading worldwide, which poses an urgent need to develop anti-SARS-CoV-2 agents. A human receptor, namely, angiotensin-converting enzyme 2 (ACE-2), supports the SARS-CoV-2 entry, therefore, serves as a target for intervention via drug. In the current study, bioinformatic approaches were employed to screen potent bioactive compounds that might be ACE-2 receptor inhibitors. The employment of a docking study using ACE receptor protein with a ready-to-dock database of phytochemicals via MOE software revealed five compounds as potent molecules. Among them, astragaloside exhibited the highest binding affinity –21.8 kcal/mol and stable interactions within the active site of the ACE-2 receptor. Similarly, the phytochemicals such as pterocaryanin B, isoastragaloside II, and astraisoflavan glucoside followed by oleuropein showed a stronger binding affinity. We hypothesize these compounds as potential lead candidates for the development of anti-COVID-19 target-specific drugs.

Keywords: ACE-2; COVID-19; molecular docking; SARS-COV-2; simulation.

1 Introduction

Coronavirus disease 2019 (COVID-19) is a highly contagious disease caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). As per the report based on the current scenario from the WHO, delivered in February cases amounting to 11, 2020, 43,103 COVID-19 have been affirmed universally, with an emerging 2,560 new case reports. In China, the number of confirmed cases reached 42,708, including 2,484 new cases, 7,333 severe cases, and 1,017 deaths [1]. Presently, no particular treatments for COVID-19 are accessible, and proper examinations aiming at the treatment of COVID-19 are missing [2].

SARS-CoV-2 is a single-stranded positive RNA virus that enters the host cell by attaching the receptor, angiotensin-converting enzyme 2 (ACE-2) [3]. The molecule ACE-2 predominantly resides on blood vessels, intestinal epithelium, and lung epithelial tissue that are crucial to the flow of blood and directing immune reactions [4]. Some studies employed protease inhibitors, lopinavir/ritonavir to treat human immunodeficiency virus patients for COVID-19 patients. Additional antiviral treatments for human pathogenic CoVs include nucleoside analogs, neuraminidase inhibitors, remdesivir, umifenovir, and lamivudine (3TC) [5]. Another examination showed nelfinavir as the best-expected inhibitor against COVID-19 Mpro, exposed via free energy computations, utilizing the atomic mechanics with generalized Born and surface area solvation (MM/GBSA) model and solvated interaction energy (SIE) methods [6].

The enormous progress in computational biology and diversified applications of bioinformatics tools have gained importance in drug designing, bringing down the time and cost needed for *in vivo* screening and experimentations that is a prerequisite for successful drug development [7, 8]. The bioinformatic approaches have substantially diminished lab trials by employing methods including identifying drug candidates, structure-based designing of the drug molecule, screening of drugs, and genome-based comparative investigation to recognize host-specific targets [9, 10].

Phytochemicals are non-nutritive plant chemicals isolated from plants. They can be carbohydrates, polysaccharides, lignin, flavonoids, alkaloids, saponin, steroids, or stilbenoids. They are natural and have protective or preventive disease properties like anti-oxidants, anti-

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bacterial, enzyme stimulators, etc., making them significant in the pharmaceutical and food industries [11, 12]. The ultimate aim of this study is to find a therapeutic bioactive compound that can target ACE2 receptors in SARS-CoV-2.

This study identified plant-based compounds that might directly inhibit ongoing 2019 novel coronavirus (2019-nCoV) causing pneumonia. This computational study employed a molecular docking approach to screen phytochemicals with the best binding and spatial affinity targeting the ACE2 receptor. The docking approach exposed Astragaloside V, pterocaryanin B, isoastragaloside II, astraisoflavan glucoside, and oleuropein, respectively, lead molecules that revealed a better docking profile compared to reference drug chloroquine phosphate. Further elucidation involves analyzing the phylogenetic components participating in the renin–angiotensin system (RAS).

2 Materials and methods

2.1 Building the compound library

The 3D structure of compounds was acquired from various databases of the MAPS database [13], PubChem [14], Zinc database [15], and MPD3 [16] PubChem in .sdf format. These were added with partial charges and energy minimized through MOE software that consequently added to the ligand database that finally includes 6000 compounds in MOE to enable docking. The chloroquine phosphate, a commercial drug well known anti-ACE-2 inhibitor, was used as a reference ligand to compare the docking results of phytochemicals.

2.2 Protein selection and preparation

The PDB structures of target COVID-19, ACE-2, were obtained from PDB with PDB ID: 1R4L. The protein was prepared by removing all heteroatoms, and subsequently, partial charges were added and energy minimized through MOE software.

2.3 Phylogeny study

To evaluate the similarity among the molecules that are components of the renin-angiotensin system (RAS) within the lungs, multiple sequence alignment (MSA) was done. The amino acid sequences of all the components were obtained from NCBI including ACE-2, dipeptidyl peptidase, ADAM metallopeptidase domain 17, Mas receptor, angiotensin II type 1 receptor, angiotensin II type 2 receptor, and in FASTA format with corresponding accession number AAH36375.1, QCD25822.1, NP_003174.3, sp|P35410.1, NP_114038.4, NP_000677.2, and AAG09466 respectively. Consequently, the sequences were subjected to a similarity scan via Clustal W followed by a similarity scan development via Clustal W, followed by developing a phylogenetic tree using aligned sequences.

2.4 Docking study

For docking, the 3D structure of ACE protonated and energy minimization was executed in MOE software and added to the database. The compounds were then docked with putative protein via molecular operating environment (MOE) [17], the triangular matcher algorithm [18] was selected, and the London dG to restore the poses. The top 10 ranked poses were generated while retaining the rigidity of receptor residues rigid. For comparative study, the chloroquine phosphate was docked against 1R4L.

2.5 Physiochemical property profile and toxicity predictions

The drug-likeliness properties of compounds were determined via a Molinspiration server that gives a prediction based on the 'rule of five' (Ro5) [19]. The pharmacokinetic properties can be indicated using the admetSAR database [20].

2.6 Molecular dynamics (MD) simulation

The docking validation was done via MD simulation using the Desmond v3.6 version. For this purpose, MD simulation was carried out using the model namely TIP3P (transferable intermolecular potential with three points) accompanied with boundary box of an orthorhombic-shaped. The stability of the protein-ligand interactions was acquired with the OPLS-2005 (optimized potential for liquid simulation) force field incorporating sodium ions [21]. The minimization step was achieved through algorithms including the steepest descent (SD) method and LBFGS of the protein-ligand system. The MD simulation was run with 100 ns via Desmond software to scrutinize the complexes of protein-ligand acquired from docking [22].

3 Results

3.1 Phylogenetic analysis

The evaluation of the phylogenetic relationship of ACE-2 with other proteins, including components of the renin-angiotensin system such as collectrin, ADAM17, Mas-R, AT1R, AT2R and DPP4 are depicted in Figure 1. The tree indicated that enzymes including Mas receptor, angiotensin II type 2 receptor, Mas receptor and angiotensin II type 1 receptor cluster to dipeptidyl peptidase which subsequently joins to ACE-2, that eventually group to ADAM metallopeptidase domain 17 forming a bigger cluster. The tree depicted that ACE-2 has distant relation with the rest of the molecules in the group. The minimum similarity of ACE2 potentiated the hypothesis of evaluating it as a target to inhibit SARS-CoV entry.

3.2 Docking studies

The docking of ACE2 protein with the library of 6000 compounds revealed sound interactions with several

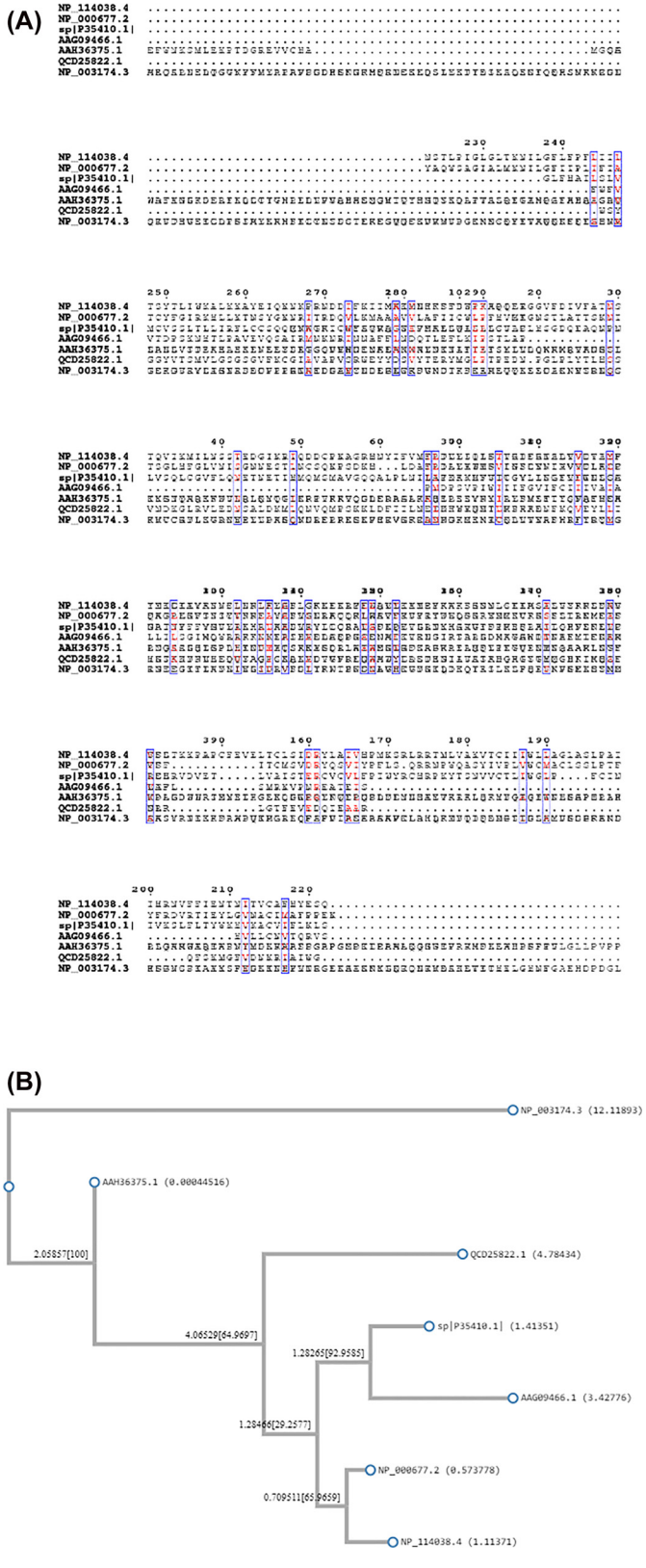

Figure 1: Phylogenetic analysis.
(A) Multiple sequence alignment of ACE2 and other enzymes involved in RAS system aligned using ClustalW 2.1 algorithm via online tool and easy sequencing in PostScript (ESPript) program was used for visualization. (B) Phylogenetic analysis was carried via ClustalW using the NJ method with (1000 repeats) bootstrap analysis and the Poisson method.

compounds. Seven compounds were chosen and selection was graded based on the highest score, RMSD value, and occupancy of sites as depicted in Table 1. The selected compounds displayed binding energy between –21.8 kcal/mol to –14.7 compared to the reference drug, which is –12.5 kcal/mol.

The diagrams generated through the LigX option of MOE tool revealed astragaloside V and pterocaryanin B

Table 1: Interaction detail of top seven bioactive phytochemicals and reference drug chloroquine phosphate in the active site of ACE2 protein.

Sr no	PubChem Id	Chemical name	Docking score (kcal/mol)	Interaction detail		
				rmsd value	Residues	Interaction
1	71,448,939	Astragaloside V	−21.8	1.6	Asn 394	H-acceptor
					Ala 346	H-acceptor
					His 505	H-donor
					Ala 99	H-acceptor
2	14,057,218	Pterocaryanin B	−21.4	1.5765	Asp 206	H-acceptor
					Ala 396	H-acceptor
3	60,148,655	Isoastragaloside II	−20.9	1.4	Ser 563	H- acceptor
					Asn 210	H-donor
					Asn 103	H- acceptor
					Arg 219	H- donor
4	46,899,140	Astraisoflavan glucoside	−15.8	1.8	His 401	H-acceptor
					Pro 346	H-donor
					His 345	Metal-ion
					Tyr 515	H-donor
					Arg 518	Metal-ion
5	5,281,544	Oleuropein	−14.7	1.7	Asn 210	pi-H
6	64927	Chloroquine phosphate	−12.5	2.0	Cys 344	H-acceptor
					Pro 346	pi-H

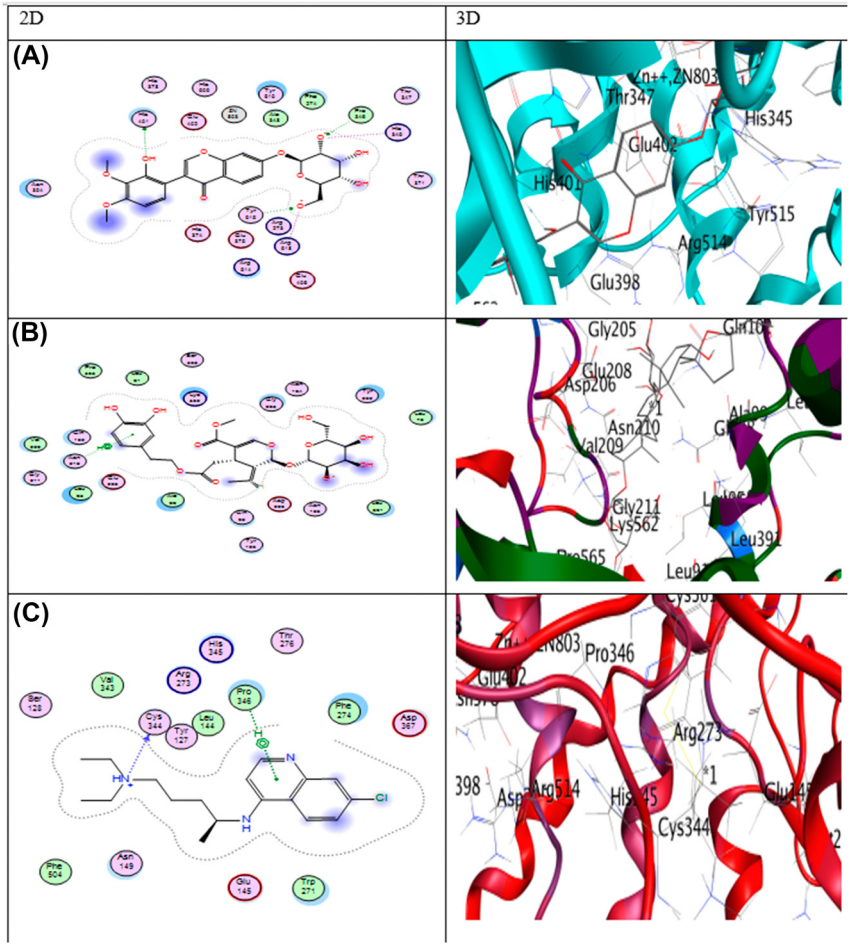

Figure 2: 2D and 3D interaction diagram of 1R4L complex with astraisoflavan glucoside (A), oleuropein (B) and chloroquine phosphate (C), respectively.

bound with ACE2 receptor with a –21.8 kcal/mol and –21.4 kcal/mol. Astragaloside V and pterocaryanin B form hydrogen bonds with the side chains of Asn 394, Ala 346, His 505, and Ala 99 (Astragaloside V) Asp 206 and Ala 396 (pterocaryanin B), respectively. The ACE2-isoastragaloside II complex displayed strong binding with Ser 563, Asn 210, Asn 103, and Arg 219 via hydrogen bonds, exhibiting a score of –20.9 kcal/mol. The oleuropein revealed arene-cation interaction with Asn 210 with a –14.7 kcal/mol score, as evident in Figure 2A. In ACE2-astraisoflavan glucoside complex, hydrogen bonding with His 401, Pro 346, and Tyr 515, and metal ion contact with residues such as His 345 and Arg 518 and a score of –15.8 kcal/mol were observed, Figure 2B. The reference ligand, chloroquine phosphate revealed to have a docking score of –12.5 kcal/mol and bound to the residue Cys 344 and Pro 346 through a single hydrogen bond and arene-cation interaction Figure 2C. The comparative study of all these ligands with chloroquine phosphate showed these compounds docked with higher energy and sound binding interactions to ACE2 receptor. The predominant bond was a hydrogen bond that implies a strong interaction of compounds with the receptor.

Table 2: Results of compounds examined for Lipinski rule.

Compound	Molecular weight (g/mol)	Number of HBA	Number of HBD	MLogP
Lipinski rule of five	<500	<10	<5	<5
Astragaloside V	943	19	8	–3.86
Pterocaryanin B	634	18	11	0.09
Isoastragaloside II	826	15	7	–1.02
Astraisoflavan glucoside	474	11	3	–1.98
Oleuropein	539.51	13	5	–3.30
Chloroquine phosphate	320.89	3	2	1.85

3.3 ADMET/drug scan results

The drug-likeness of the compounds was predicted through the molinspiration server, based on the Ro5 (Table 2). Although all the candidates show affinity with the target protein TRIM59, two out of the top seven compounds fulfilled all the requirements of the Lipinski rule, suggesting that they can be the potential inhibitor of ACE2. These two compounds include astraisoflavan glucoside and oleuropein. All the chosen compounds were assessed for pharmacokinetic properties through the admetSAR server for drug-likeliness which showed a sound safety profile of these compounds (Table 3).

3.4 MD simulation

MD simulation was carried out following the docking measurements with the Desmond Simulation Package for 100 ns per complex. MD trajectories calculated the RMSD values, the root mean square fluctuation values, and the protein-ligand interaction values. To test the MD simulation

Table 3: ADMET profiling enlisting absorption, metabolism, and toxicity related drug-like parameters of selected compounds.

A. ADMET Profiling						
Compounds	**Astragaloside V**	**Pterocaryanin B**	**Isoastragaloside II**	**Astraisoflavan glucoside**	**Oleuropein**	**Chloroquine phosphate**
A. Absorption						
Blood–brain barrier	+	–	+	–	+	+
Gastro-intestinal absorption	+	+	–	+	+	+
P-glycoprotein substrate	Yes	No	Yes	No	Yes	Yes
B. metabolism						
CYP450 1A2 inhibitor	No	No	No	No	No	No
CYP450 2C9 inhibitor	No	No	No	No	No	No
CYP450 2D6 inhibitor	No	No	No	No	No	No
CYP450 2C19 inhibitor	No	No	No	No	No	No
CYP450 3A4 inhibitor	No	No	No	No	No	No
Distribution						
Subcellular localization	Mitochondria	Mitochondria	Mitochondria	Mitochondria	Mitochondria	Lysosomes
Toxicity						
AMES toxicity	No	No	No	No	No	No

outcome, we conducted MD simulations with similar parameters for each compound with highly reproducible results. The stability of the modeled proteins was verified by plotting the root mean square deviation (RMSD) during the production run. The RMSD for the complex of ACE2 with ligand oleuropein showed the fluctuation of 0.5 nm and the stable trajectory throughout the production run with maximum deviation in the range of 1.75 Å and 2.25 Å (Figure 3A). The RMSD plot for the complex of ACE2 with ligand astraisoflavan glucoside showed the fluctuation maxima at up to 75 ns and acquired stability beyond and before the 75 ns simulation interval and RMSD value was between 1.00 and 2.15 Å for both protein and ligand (Figure 4A). The flexibility of individual amino acid residues of the complexes was ascertained via RMSF analysis. The ACE2-oleuropein complex had an RMSF value between 0.7 and 4.0 Å with the local ligand-contact fluctuation at several points throughout at 2.3 Å with the intense movement of 4.0 Å between residues no. (100–150) depicted in (Figure 3B). Several fluctuations were observed for the complex ACE2-astraisoflavan glucoside, with the local ligand-contact maxima at 3.5 Å at residue 110 Å (Figure 4B). However, RMSF displayed acceptable convolutions regarding the atoms of protein and ligand.

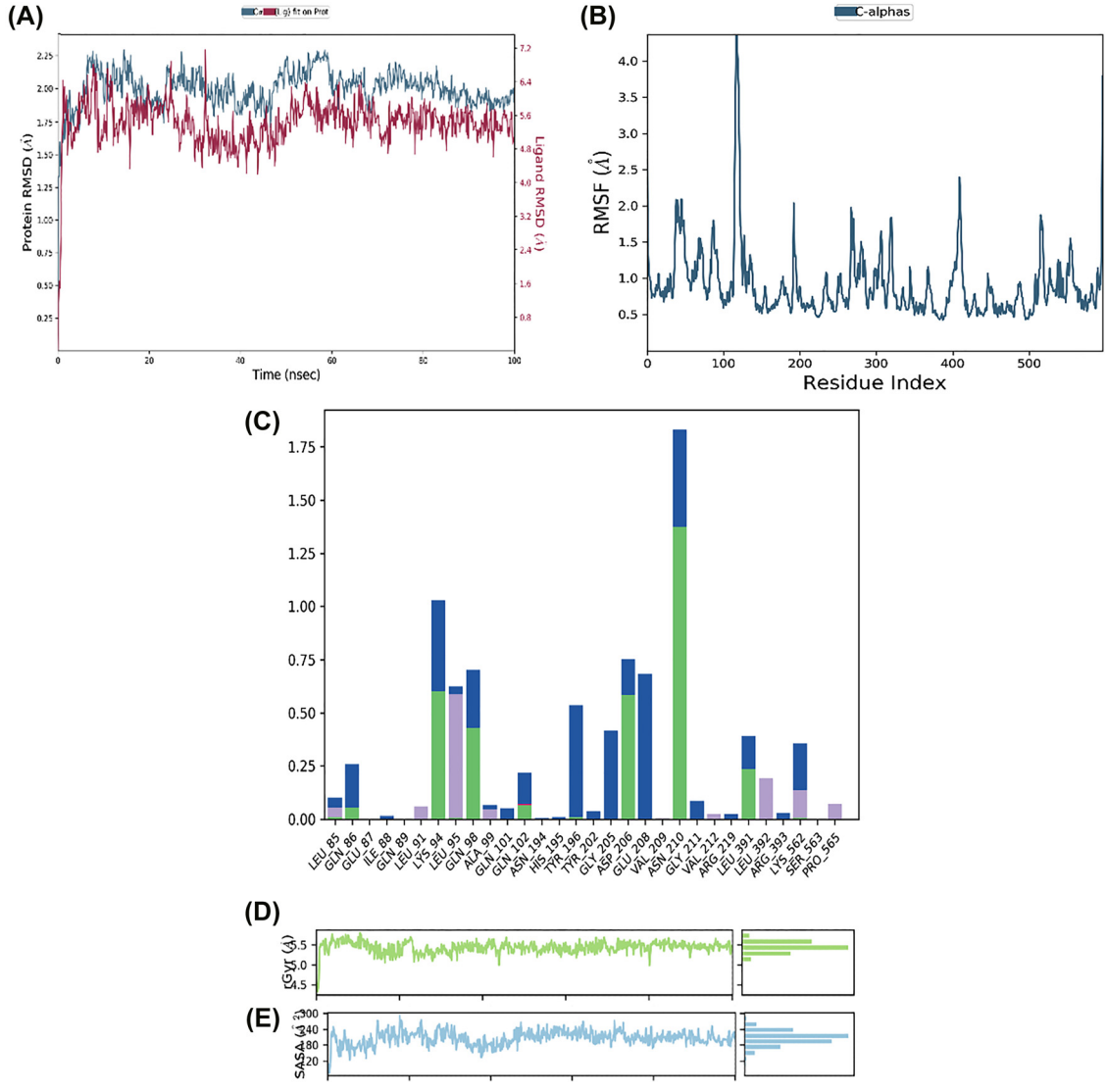

Figure 3: MD simulation interaction diagrams of 100 ns trajectory showing (A) root mean square deviation; (B) root mean square fluctuation of residue with viable ligand contacts; (C) protein-ligand contacts histogram; (D) radius of gyration of protein; (E) SASA for oleuropein-ACE2 complex.

The contact profiles of complexes were computed from the trajectories as shown in Figures 3C and 4C. The compound oleuropein interacted with TYR_196, GLU_205, GLU_208, and Asn_210 by forming a water bridge. The residues, including ASN_210, LYS_94, and ASP_206 contributed to the H-bond interactions. The LEU_95 displayed hydrophobic interaction (Figure 3C). Astraisoflavan glucoside, ligand interacted with PHE_40, TRP_349, and TYR_510 through hydrophobic interaction, and two residues such as THR_371 and HIS_374 involved in water bridge formation. Moreover, a hydrogen bond was formed by the residues GLU_375 and GLU_402 (Figure 4C).

Further, the parameter such as radius of gyration (Rg) for the complex oleuropein-ACE2 complex calculated through the simulation of 100 ns was 3.4 Å ± 2.7Å and for ACE2-astraisoflavan glucoside complex was 4.1 Å ± 3.8 Å as depicted in Figures 3D and 4D. The trajectory in the course of the simulation run was stable, suggesting

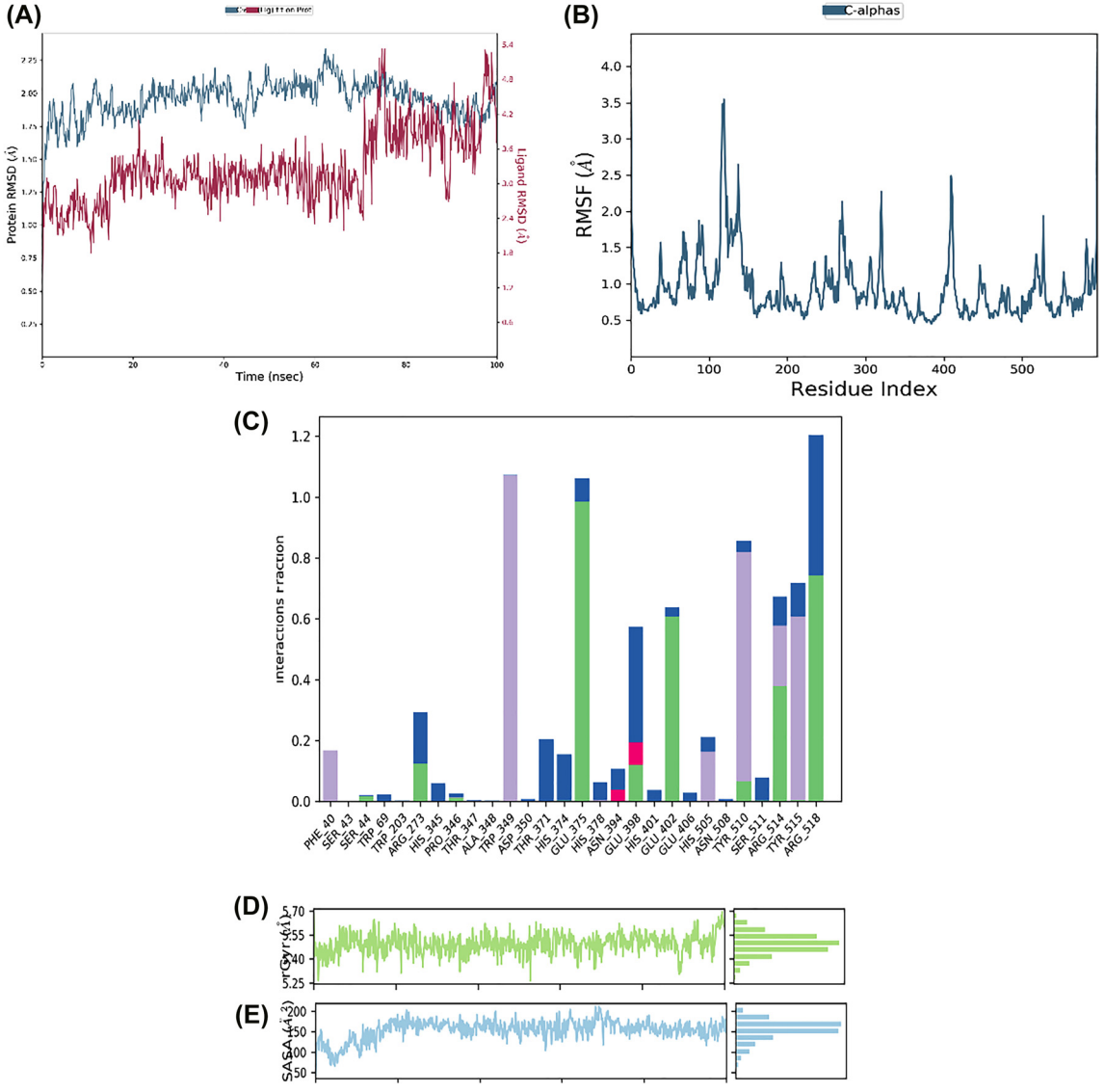

Figure 4: MD simulation interaction diagrams of 100 ns trajectory showing (A) root mean square deviation; (B) root mean square fluctuation of residue with viable ligand contacts; (C) protein-ligand contacts histogram; (D) radius of gyration of protein; (E) SASA for astraisoflavan glucoside-ACE2 complex, respectively.

sound compactness in interaction. SASA values were computed to estimate the H_2O accessibility surface area that provides information regarding the alteration in conformations acquired from dynamic interactions. The Solvent accessible surface area (SASA) ascertained for oleuropein-ACE2 and ACE2-astraisoflavan glucoside complex for 100 ns was 0.1 ± 0.3 nm^2 and 0.1 ± 0.4 nm^2, respectively (Figures 3E and 4E).

4 Discussion

The COVID-19 pandemic is as yet progressing, so it is dire to discover new preventive and restorative specialists as quickly as time permits. Undoubtedly, explicit immunizations and antiviral medicines are the best techniques to forestall and treat viral disease, and there are not yet successful medicines that focus on the 2019-nCoV. The antiviral agents against corona may need months or years, implying that the quickest treatment is required. The current study involves applying a molecular docking approach to the ACE receptor. Briefly, a database of 6000 phytochemicals was made and imported to MOE after processing that involved protonating and minimizing. It was followed by docking with the ACE receptor that facilitated the entry of corona within the host cells.

Various studies demonstrated that cells lacking ACE2 are escaped safely and no SARS-CoV-2 can enter, apart from other receptors including aminopeptidase N and dipeptidyl peptidase 4 [23]. Thus, novel compounds need better inhibitory activity against ACE protein, thereby inhibiting the entry of the SARS virus. The ACE receptor was docked with a library of phytochemicals that revealed five potent common phytochemicals including astragaloside V, pterocaryanin B, isoastragaloside II, astraisoflavan glucoside, and oleuropein. However, it demands opportunities for the further optimization of the compounds through experimental study. It is needed to figure out the phytochemicals that combat the effect of COVID-19 (2019-nCoV). The release of secondary metabolites in tropical plants might be utilized as medicinal prescriptions [24, 25]. Several compounds, like flavonoids, from therapeutic plants, have been accounted for to have biological activities as antivirals [26, 27]. The discoveries of the current examination will furnish different aspects with freedoms to recognize the suitable medication to battle COVID-19 subjected to experimental evaluation.

Further, we have exploited the similarity index of ACE2 with ACE, Mas-R, ADAM17, AT1R, AT2R, collectrin, and DPP4. These were considered multiple sequence alignment owing to their significance in the RAS system. Apart from this, both carboxypeptidase viz., collectrin, and ACE2 protein are homologs [28]. The DPP4 holds importance as the MERS-CoV receptor [29]. Intriguingly, the lung damage resulting from infection by this virus is ascribed to the ACE-2 receptor present in the lungs, which is pivotal to the RAS system [30].

It has been reported in multiple studies that angiotensin II contributed essentially to the development of cardiovascular hypertrophy. ACE inhibitors intervene with angiotensin II and angiotensin I formation and delimit bradykinin catabolism. Both approaches interfere with the cardiovascular hypertrophy of hypertension [31]. Our analysis revealed a distant relationship of ACE-2 with seven enzymes in the chain of RAS.

The docking and simulation have been executed on numerous natural compounds such as extract of *Ocimum sanctum* on protease as a target [32]. Apart from this, RBD from spike and protease from SARS-CoV-2 was used as a target against brazilin, curcumin, and galangin from *Curcuma* sp., *Citrus* sp., *Caesalpinia sappan, and Alpinia galanga* [33].

In the current study, docking followed by the simulation approach highlighted the significance of hit compounds such as oleuropein and astraisoflavan glucoside in deciphering the ACE-2 inhibitory activity. The residues participated with exceptional binding affinity against ACE-2 with involved residues such as TYR_196, GLU_205, GLU_208, ASN_210, LYS_94, ASP_206, PHE_40, TRP_349, TYR_510, THR_371, HIS_374, GLU_375, and GLU_402.

A patent by the U.S. stated Ole to possess significant antiviral potency against numerous viruses including bovine rhinovirus, rotavirus, mononucleosis by herpes, hepatitis, and feline leukemia virus [34, 35]. Intriguingly, the antiviral effect of Ole has also been demonstrated against parainfluenza type 3 and respiratory syncytial virus [36]. Additionally, the flavonoid or plants enriched in flavonoids is widely stated as beneficial against corona symptoms [37–39] and exhibited immunomodulatory and antiviral responses against coronavirus [40].

Overall, the docking and simulation outcomes were reproducible enough to report strong inhibition against ACE-2. The parameters examined in the simulation run displayed steadiness in confirmation. The safety profile of the said hit compounds is evident from the pharmacokinetic analysis carried out in the study.

5 Conclusion

In conclusion, phytochemicals screening against ACE-2 reported astragaloside V, pterocaryanin B, isoastragaloside II, astraisoflavan glucoside, and oleuropein as potent molecules against ACE-2. These can serve to alleviate the consequences of SARS-CoV. However, the study demands further exploration via experimental and preclinical studies.

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Author contributions: UAA generated the idea of the detailed research work, designed and performed molecular docking work, and reviewed the draft. HK performed the docking and ADMET analysis, ME helped HK in silico work, SK revised the draft. MS performed simulation.

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Data Availability: All data and materials generated or analyzed during this study were included in the published article.

Code availability: Not applicable.

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