A molecular dynamics simulation study of the ACE2 receptor with screened natural inhibitors to identify novel drug candidate against COVID-19

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Background & objectives: The massive outbreak of Novel Severe Acute Respiratory Syndrome Coronavirus (SARS-CoV-2) has turned out to be a serious global health issue worldwide. Currently, no drugs or vaccines are available for the treatment of COVID-19. The current computational study was attempted to identify a novel therapeutic inhibitor against novel SARS-CoV-2 using *insilico* drug discovery pipeline. **Methods:** In the present study, the human Angiotensin-converting enzyme 2 (ACE2) receptor was the target for the designing of drugs against the deadly virus. The 3D structure of the receptor was modeled & validated using Swiss-model, Procheck & Errat server. A molecular docking study was performed between a group of natural & synthetic compounds having proven anti-viral activity with ACE2 receptor using Autodock tool 1.5.6. The molecular dynamics simulation study was performed using Desmond *v 12* to evaluate the stability and interaction of the ACE2 receptor with a ligand. **Results:** Based on the lowest binding energy, confirmation, and H-bond interaction, cinnamic acid (-5.20 kcal/mol), thymoquinone (-4.71 kcal/mol), and andrographolide (Kalmegh) (-4.00 kcal/mol) were screened out showing strong binding affinity to the active site of ACE2 receptor. MD simulations suggest that cinnamic acid, thymoquinone, and andrographolide (Kalmegh) could efficiently activate the biological pathway without changing the conformation in the binding site of the ACE2 receptor. The bioactivity and drug-likeness properties of compounds show their better pharmacological property and safer to use. **Interpretation & Conclusions:** The study concludes the high potential of cinnamic acid, thymoquinone, and andrographolide against the SARS-CoV-2 ACE2 receptor protein. Thus, the molecular docking and MD simulation study will aid in understanding the molecular interaction between ligand and receptor binding site, thereby leading to novel therapeutic intervention. **Keywords:** SARS-CoV-2, ACE2 receptor, Natural compounds, Molecular Docking, Molecular Dynamics Simulation, Drug Discovery.

1 **Molecular Dynamics Simulation Study of ACE2 Receptor with Screened**
2 **Natural Inhibitors to Identify Novel Drug Candidate against COVID-19**

3 **Abstract**

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33 Dynamics Simulation, Drug Discovery.

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1. Introduction

Novel Severe Acute Respiratory Syndrome Coronavirus (SARS-CoV-2) is a unique virus with an unusually large positive-sense RNA genome ranging from o 32kb in length [1] and a special replication strategy. It is a member of the largest group of viruses, Nidovirales [2]. It is enveloped and characterized by club-like spikes that project out from the surface. The receptor-binding domain of spike protein binds with the host cell and makes the virus capable of causing a variety of diseases in the host [3]. It causes enteritis in cows and pigs and upper respiratory disease in birds. It is now proven to cause potentially lethal respiratory infections in humans. Before the outburst of the coronavirus, it was thought that these viruses cause only mild, respiratory infections in humans but after the outburst, lethal side effects of the virus have become the major global concern. These viruses are known to cause more severe diseases in neonates, the elderly, and individuals with underlying illnesses [4].

In December 2019, Wuhan, China became the center of the outburst of the novel SARS-CoV-2 virus which raised intense and urgent concern internationally. A similar outburst of the SARS-CoV virus was also experienced in 2003 in Guangdong, China. The number of cases at that time increased substantially and spread globally [5]. In 2003, the virus-infected approximately 8000 people and caused 774 deaths worldwide [6]. The major reason for the spread of SARS-CoV in 2003 was the ability of the virus to transmit rapidly among people. Also, the insufficient preparedness and implementation of control measures lead to the further spread of the virus. Learning from the past experiences, isolation of people suspected to have the disease, close monitoring of their contacts, epidemiological and clinical data collection from patients, and development of diagnostic as well as treatment procedures are some of the widely accepted measures adopted to control the spread of novel coronavirus [7]. The 2019 outbreak of novel SARS-CoV-2 has infected more than 66,818,411 people with 1,428,870 deaths worldwide as of November 2020. The control of the spread of the virus and also an adequate plan of action and treatment of the virus is the major need of the hour. Therefore, researchers are now trying to find a drug that can considerably treat the effects of the virus. In the present study, we aim to identify compounds that can be used as prophylactics against novel SARS-CoV-2. Coronavirus contains a genome of approximately 30kb and consists of a 5' cap structure and 3' poly(A) tail that allows it to act as mRNA for the translation of the replicase polyproteins. At the beginning of each structural or accessory gene, there is the presence of transcriptional regulatory sequences (TRSs) which are essential for the expression of each of these genes. SARS-CoV-2 is known to bind with angiotensin-converting enzyme 2 (ACE2) as its receptor to gain entry into the human cells [8]. Various non-structural proteins assemble into the replicase- transcriptase complex of the viral genome to provide an environment suitable for the synthesis of RNA and also contain enzyme domains and functions which are important for the replication of RNA. Various non-structural proteins (nsp) play an important role in spreading and activating the virus inside the host. Nsp1 promotes cellular mRNA degradation and blocks host cell translation, which ultimately results in the blockage of the innate immune response [9,10]. Nsp2 binds to

76   prohibition proteins and further enhances the spread of the virus in the body [11]. Nsp3 is a
77   large, multi-domain transmembrane protein consisting of Ubl1 and Ac domains that interact with
78   N protein, ADRP activity that promotes cytokine expression, and PLPro/ Deubiquitinase domain
79   that cleaves viral polyprotein and blocks host innate immune response [12-14]. Nsp4 and nsp6
80   are potential transmembrane scaffold proteins, important for the proper structure of DMVs
81   [15,16]. Nsp5 cleaves viral proteins, nsp7, and nsp8 act as a clamp for RNA polymerase [17]
82   nsp9 is an RNA binding protein, and nsp13 acts as RNA helicase for the viral genome. Nsp10
83   acts as a cofactor for nsp14 (an important protein for proofreading of the viral genome [18]) and
84   nsp16 (shields viral RNA from MDA5 recognition [19]) and stimulates ExoN and 2-0-MT
85   activity [20,21].

86   Novel SARS-CoV-2 is very similar to SARS-CoV [22-24]. Both these viruses belong to the
87   betacoronavirus genus (lineage B) [25,26]. The detailed comparison of SARS-CoV, MERS-CoV,
88   and SARS-CoV-2 is depicted in table 1 [27].

89   The entry of the virus into the cell is one of the most primary and essential steps for the spread of
90   disease caused by novel SARS-CoV-2. This virus encodes a surface glycoprotein known as spike
91   [28]. The receptor-binding domain (RBD) of the spike protein is responsible for binding with the
92   host cell receptor and mediates the entry of the virus into the cell. The virus enters the host cell
93   due to the release of spike fusion peptide formed as a result of cleavage of spike protein by the
94   host protease [29,30]. The similarity in the receptor-binding domain of both SARS-CoV-2,
95   suggests that both these viruses share a common receptor, ACE2. Angiotensin-converting
96   enzyme 2 (ACE2) is primarily found in alveolar epithelial cells [31] and is responsible for
97   reducing the surface tension of these cells, thereby preventing the collapse of alveoli. Therefore,
98   ACE2 is a very important protein to ensure proper gas exchange through the lungs. Any injury
99   caused in such cells will lead to severe lung injury, which was commonly observed in COVID-
100 19 patients. The binding of the spike protein of SARS-CoV-2 with ACE2 of host cell leads to
101 endocytosis of virus and loss of ACE2 function, therefore due to this binding, the cell loses its
102 main component which was responsible for protecting the lungs from any injury. This makes the
103 host vulnerable to viral infection. The receptor-binding domain of the spike glycoprotein binds to
104 the tip of subdomain 1 of ACE2. The viral membrane fuses with the host cell and activates the
105 cell [32-34]. After fusion, viral RNA is released into the cytoplasm that establishes infection.
106 Thus, targeting the ACE2 receptor of host cells can block the entry of the virus into the cell,
107 thereby protecting the host from viral infection and pandemic disease COVID-19.

108 Ever since the outburst of the pandemic, researches are going on worldwide, to explore different
109 aspects of SARS-CoV-2 and the mechanism of infection. From repurposing drugs to finding new
110 drugs and vaccine candidates, the studies on SARS-CoV-2 have come a long way. Some of the
111 recent studies explore new immunogenic dimensions of the pandemic. In one of the studies, the
112 researchers took the ontological approach to identify targets, both in the virus and the host, that
113 can aid in searching for effective vaccines and drugs against COVID-19 [35]. In another study,
114 researchers conducted molecular modeling intending to reveal new druggable binding sites in the

115 spike protein of SARS-CoV-2. In their study, they disclosed 8 novel druggable binding sites of
116 spike protein [36]. In one of the studies, the scientists tested the potential of flavonols to act as
117 antiviral drugs by targeting spike protein, SARS-CoV-2 proteases, RNA-dependent RNA
118 polymerase, and ACE2 receptor [37].

119 Therefore, there is a need for more effective and rational approaches that open the door to the
120 development of more effective new therapeutics targets and drug candidates against Covid-19. In
121 this regard, computational approaches play a significant role in the process of rapid drug
122 discovery and development process with less time and cost-effective manner. Besides this, herbal
123 medication shows a hugely significant impact on human health [38]. Nearly about 70-80% of the
124 world population relies on herbal drugs due to their great compatibility with lower/zero side-
125 effects on human health. The bio-active constituents present in the plants possess medicinal
126 properties is used by various pharmaceutical and R & D industries for the development of drug
127 due to their safer and better use [39]. In the current *insilco* study, drug discovery approaches are
128 implemented to identify novel therapeutic inhibitors against novel SARS-CoV-2 targeting the
129 human Angiotensin-converting enzyme 2 (ACE2) receptor.

130

131 **2. Methodology**

132 **2.1. Protein Structure Prediction and Validation**

133 The protein sequence of the ACE2 receptor was retrieved in FASTA format from the NCBI
134 public database. For the identification of similar sequences, the BLAST program was performed
135 for the search of structurally similar sequences with a protein databank database (PDB) [40]. The
136 3D structure of the ACE2 receptor was modeled using online modeling server SWISS-Model
137 [41] based on the BLAST parameter. Further, the energy minimization of the modeled structure
138 was done using Chimera 1.10.1 tool [42]. The 3D structure was subjected to validation using
139 Procheck and Errat Plot. The Ramachandran plot [43] evaluates any steric clashes and structure
140 reliability whereas the errat plot measures the overall error frequency rate in modeled structure.

141 **2.1. Ligand Identification**

142 The Thirty natural & synthetic compounds having proven *in-vitro* & *in-vivo* anti-viral activity
143 was used in the study based on various literature reviews are depicted in table2 & table3 [44].
144 The available 2D & 3D structures of compounds were retrieved from the PubChem database.
145 Molinspiration chemoinformatics software was used to predict the Molecular and bioactivity
146 analysis as well as drug-likeness properties of these compounds.

147 **2.3. Molecular Docking Study**

148 A molecular docking study was performed between the ACE2 receptor and 30 natural &
149 synthetic compounds using the Autodock 1.5.6 tool to predict the best binding score, affinity,
150 and confirmation [45,46]. AutoDock is an automated suite of protein-ligand docking tools used

151 to predict the protein interactions with small molecules such as drug molecules and substrate. It
152 analyzes the interaction of ligand molecules at the specified target site of the protein. AutoDock
153 uses AMBER forcefield and linear regression to predict the free binding energies [47]. The
154 lowest binding energy was the criteria undertaken to select the best protein-ligand complex.
155 Calculation of binding energy was performed using a semi-empirical free energy force field with
156 charge-based desolvation and grid-based docking. The force field was selected based on a
157 comprehensive thermodynamic model that allows the incorporation of intermolecular energies
158 into the predicted binding energy [48]. Cygwin platform was used to run docking files.
159 Discovery Studio 4.5 was used to visualize and analyze protein-ligand interaction and
160 confirmation.

161 **2.4. Molecular Dynamic Simulation Study**

162 The molecular dynamic simulation study was performed to examine the conformational changes
163 in the protein that occurred due to the ligand-binding site and to evaluate the effect of these
164 changes over the protein-ligand complex [49, 50]. To evaluate the stability and interaction of the
165 ACE2 receptor with ligand simulation study was performed using Desmond *v12* Schrödinger
166 software package at 50 ns time period [51]. The complex was placed in protein preparation
167 wizard for optimization, analysis, and refinement of docking complex, system builder menu set
168 up membrane model POPC (Palmitoyl Oleoyl Phasphatidyl chlorine). Water molecules were
169 added to the docking complex of the ACE2 receptor with a simple point charge (SPC) water
170 model. System builder was build using counter ions, shake algorithm used to constrain the
171 geometry of water molecules, and heavy atom bond lengths with hydrogen, electrostatic
172 interaction applied using Particle Mesh Ewald (PME) method and Orthorhombic were used as
173 periodic boundary conditions (PBC). The energy was minimized with 5000 steps maximum
174 iteration using the steepest descent algorithm (SD) and 1000 steps using the conjugate- gradient
175 algorithm (CG) with convergence threshold 50 e. Dynamic was performed with 50 ns, during the
176 simulation the length of bond involving hydrogen was constrained using NPT ensemble, without
177 restraints, for a simulation time of 1.2 picoseconds (ps) (temperature 300K) was performed to
178 relax the system.

179 **3. Result**

180 **3.1. 3D Structure Prediction & Validation**

181 The total length of the ACE2 receptor in humans is 805 amino acids. The 3D model of protein
182 was modeled using the online modeling server SWISS-Model. Based on the lowest z-score and
183 valid q-mean score the best model was predicted. The modeled 3D structure was subjected to
184 validation using Procheck and Errat Plot. The Ramachandran plot was used to evaluate the
185 position of amino acid residues in the allowed and disallowed region and the overall
186 stereochemical property of the protein structure. Errat plot was used to calculate the overall error
187 frequency rate. The Ramachandran plot showing 90.9% in the favored region; indicate good
188 structure stability and reliability. The errat plot showing quality factor 96.08% reveals non-

189 bonded interaction with different atoms in the modeled structure which indicates good quality of
190 the model (Fig1).

3.2. Molecular Docking Study

192 Molecular docking study is known to be the most reliable method to analyze and predict the best
193 fit protein-ligand confirmation [52, 53]. A molecular docking study was performed between
194 ACE2 receptor and thirty compounds using Autodock 1.5.6 tools to obtain a higher stable
195 protein-ligand complex (Table4). Based on the lowest binding energy (ΔG_b, kcal/Mol),
196 confirmation, hydrogen bond interaction, the three compounds namely Cinnamic acid(-5.20
197 kcal/mol), Thymoquinone(-4.71 kcal/mol), and Andrographolide(Kalmegh) (-4.00 kcal/mol)
198 were screened out showing strong binding affinity to the active site of ACE2 receptor (Fig2 &
199 Table 5). Thus these compounds may be considered as a suitable therapeutic inhibitor against
200 SARS-CoV-2. Further, all three dock complexes were subjected to a molecular dynamics
201 simulation study to check the stability of protein-ligand complexes.

3.3. Molecular Dynamic Simulation study

203 MD simulation study was performed to check the stability of the ACE2 receptor with cinnamic
204 acid, thymoquinone, and green chireta using Desmond *v12* based on docking results and h-bond
205 interaction study. To study the stabilities, root means square deviation (RMSD) was calculated
206 with respect to the initial structures along the 50 ns (ns) each trajectory with average RMSD
207 (Root mean square deviation) 12.0A – 4.5A for ACE2-cinnamic acid complex (Fig.3), 12.0A –
208 6.0A for ACE2-thymoquinone complex (Fig.4), and 12.0A – 6.0A for ACE2-
209 Andrographolide(Kalmegh) (Fig5). In summation, complex stability also proved the validity of
210 the docking results. There is no effect of temperature and pressure on the conformation of the
211 structure.

4. Discussion & Conclusion

213 The causative agent of COVID-19 respiratory disease, novel coronavirus SARS-CoV-2
214 pandemics is occurring as a life-threatening disease infecting more than 66,818,411 people
215 worldwide. Nearly about 1,428,870, total deaths estimated so far. At current, there are no
216 available antiviral drugs or vaccines with proven efficacy for the prevention and treatment of the
217 diseases. Therefore, a more effective and rational approach is needed that will ultimately lead to
218 new therapeutic approaches. In this regard, the computational approach holds promising trends in
219 the drug discovery & development process with reducing cost and time. In the current *insilico*
220 study, the angiotensin-converting enzyme 2 (ACE2) receptor was taken as the target protein as
221 various literature studies suggested that ACE2 as a functional SARS-CoV-2 receptor required for
222 host cell entry and subsequent viral replication [54]. The 3D structure of the ACE2 receptor was
223 modeled and validated. A docking study of filtered natural & synthetic compounds was
224 performed with the ACE2 receptor. Based on the lowest binding energy, docking confirmation,
225 and H-bond and pi-pi interaction Cinnamic acid (-5.20 kcal/mol), Thymoquinone (-4.71
226 kcal/mol), and Andrographolide (Kalmegh) (-4.00 kcal/mol) showing strong binding at the active

227 site of ACE2 receptor. Further, The MD simulation result reveals that cinnamic acid,
228 thymoquinone, and andrographolide could efficiently activate the biological pathway without
229 changing the conformation in the binding site of the ACE2 receptor. The bioactivity and drug-
230 likeness properties of compounds show their better pharmacological property and safe to use.
231 Cinnamic acid derived from the plant *Bromeliad,* have a virucidal activity, involved in the
232 inhibition of the viral replication cycle including the formation of the viral envelope and cell
233 membrane thereby affecting the whole epidemiology of the viral protein. It is known to be an
234 excellent phytochemical that have cytopathic activities against all viral infections. The study
235 reported [55] the potential of plant extracts and natural compounds against SARs CoV-2 and
236 other deadly viruses showed the suppressive effect of *trans*-cinnamic acid (IC50 = 3.0 ± 0.18
237 µg/ml, SI = 7.4) on Covid-19 infection. The molecular docking study conducted on Vero cell
238 against SARS CoV-2 viral infection targeting Mpro protein showed the highest activity with
239 cinnamic acid [56]. Thymoquinone derived from the plant *Nigella sativa*, significantly enhances
240 immune responsiveness and suppresses pathogenicity of many viruses. . The study suggested that
241 it blocked the replication of SARS-CoV spike (S) protein, which plays a major role in host cell
242 viral attachment to receptor angiotensin-converting enzyme 2 (ACE2). The study reports the
243 antinociceptive effects of thymoquinone produce via indirect activation of supraspinal µ1- and κ-
244 opioid receptors [57]. In reference to this hemorphins, opoid active peptide also block the effect
245 of ACE [58]. Thus these findings suggest a similar inhibitory molecule for both opoid receptor
246 and ACE2. Therefore, thymoquinone might be proved as a novel inhibitory molecule for ACE2
247 in SARS-CoV-2. In an *in-Silico* study [59] targeting E protein of SARs CoV-2 shows good
248 binding affinity and confirmation (BE= -9.01 kcal/mol) with thymoquinone using   Argus Lab
249 4.0. Further, their comparative study with patch dock also showed better affinity (BE= -32.03
250 kcal/mol). Thus these findings suggest the effectiveness of thymoquinone against SARsCoV-2
251 targets. In the study on a natural compound having involvement in ER stress against HSPA5
252 substrate-binding domain β (SBDβ), that is known to be on recognition site of SARsCOv-2 spike
253 show average binding energy -6.25 ± 1.10 and -5.520 ± 0.12 kcal/mol to with thymoquinone
254 [60]. Therefore it is probably used as an anti-covid-19 drug for the patient having pre-existing
255 medical condition or stress. Green chiretta or Kalmegh (Andrographis paniculata) is an excellent
256 medicinal plant having anti-HIV, anti-pathogenic, anti-viral, and immunoregulatory activities. Its
257 bioactive constituent andrographolide has been reported to have immunoregulatory activities,
258 plays a significant role as a modulator of altered immune responses. It can regulate both classical
259 and alternative activation of macrophages, and specific antibody production as well as antigen-
260 specific producing splenocytes. The insilico study [61]conducted to evaluate the potential of
261 Andrographolide against covid-19 infection showed high binding energy, better confirmation,
262 bioactivity, and pharmacodynamics property against main protease (Mpro) protein of SARS-
263 CoV-2. To study the potential of andrographolide (AGP1), 14-deoxy 11,12-didehydro
264 andrographolide (AGP2), neoandrographolide (AGP3), and 14-deoxy andrographolide (AGP4),
265 molecular dynamic and simulation study were performed against the central targets of virus
266 namely non-structural proteins (3 L main protease (3CLpro), Papain-like proteinase (PLpro) and

267 RNA-directed RNA polymerase (RdRp)) and a structural protein spike protein (S). The finding
268 suggests the effectiveness and potential of AGP3 analog against covid-19 infection [62]. An
269 integrated study on liquid chromatography-tandem mass spectrometry (LC-MS/MS)
270 metabolomics and network pharmacology on kalmegh suggested its active role as immune-
271 protective and antiviral activity as well as its involvement in various pathway such as toll-like
272 receptor pathway, PI3/AKT pathway, and MAP kinase pathways against SARs CoV-2 [63].
273 Thus, this phytochemical can inhibit viral propagation and can act as an excellent antiviral drug.
274 Therefore, the obtained compounds if synthesized and tested on an animal model will hold
275 promise in the treatment of the SARS CoV-2 virus.
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279 **5. Conclusion**
280 The study concludes the high potential of Cinnamic acid, Thymoquinone, and Andrographolide
281 against SARS-CoV-2 ACE2 receptor protein. The molecular docking & molecular dynamics
282 simulation study reveals the better binding affinity, stability, and structural conformation at the
283 binding site of these compounds against the ACE2 receptor. Therefore, the above finding will
284 help to design a novel potent therapeutic inhibitor against SARS-CoV-2.
285

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292

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485

Comparison_between_SARS-CoV,_MERS-CoV,_and_SARS-CoV-2..docx

1   **Table1.** Comparison between SARS-CoV, MERS-CoV, and SARS-CoV-2.

Characteristic	SARS-CoV	MERS-CoV	SARS-CoV-2
Year in which the first case was reported	2002	2012	2019
Country/region where the first case was reported	China	Middle East	China
The primary mode of transmission	Droplets, aerosol, and contact	Droplets, aerosol, and contact	Droplets, aerosol, and contact
Incubation period	2-7 days	2-14 days	2-14 days
Host receptor	ACE2	DPP4	ACE2
Case fatality rate	Approximately 15%	34.4%	1-3%

List_of_natural_Compound.docx

1 **Table2.** List of natural compounds and their chemical properties used for study.

S.No.	Compound Name	Plant derived	Mol. wt(g/mol)	H-bond donor	H-bond Acceptor
1.	Choline	*Withania Somnifera*	104.17	1	2
2.	Harmine	*Passiflora Incarnata*	182.23	1	2
3.	Cinnamaldehyde	*Cinnamonum Cassia*	132.16	0	1
4.	Cinnamic Acid	*Bromeliad*	148.16	1	2
5.	Coumarins	*Dypsis Lutescens*	146.15	0	2
6.	Ursolic Acid	*Ocimum Basilicum*	456.7	2	3
7.	Chlorogenic Acid	*Daucus Carota*	354.31	6	9
8.	Assafoetidnol B	*Ferula Assafoetida*	456.54	2	7
9.	Glucobrassicin	*Brassica Oleracea*	448.48	6	11
10.	Linamarin	*Phaseolus Lunatus*	247.25	4	7
11.	Luteolin	*Thymus Vulgaris*	286.24	4	6
12.	Andrographolide(Kalmegh)	*Andrographis Paniculata*	350.4	3	5
13.	Catechin	*Camellia Sinensis*	458.4	15	12
14.	Citral	*Melissa Officinalis*	152.24	0	1
15.	Hydroxychloroquine	*Synthetic*	335.9	2	4
16.	Linalool	*Lavandula Angustifolia*	154.25	1	1
17.	Nicotine	*Nicotiana Tabocum*	162.23	0	2
18.	Chloroquine	*Helianthus Annus*	319.9	1	3
19.	Allium	*Allium Sativum*	150.3	0	0
20.	Thymoquinone	*Nigella Sativa*	164.2	0	2

List_of_synthetic_compounds.docx

1 **Table3.** List of synthetic compounds and their chemical properties used for study.

S.No.	Compound Name	Mol. wt (g/mol)	H-bond donor	H-bond Acceptor
1.	Amprenavir	505.64	1	4
2.	Acyclovir	225.21	4	8
3.	Umifenovir	477.42	1	5
4.	Combivir	505.5	5	18
5.	Tamiflu	312.41	3	6
6.	Zanamivir	332.31	9	11
7.	Cidofovir	279.19	5	9
8.	Peramivir	328.41	7	8

Molecular_docking_result.docx

1   **Table4.** Result of molecular docking study with natural and synthetic compounds

S.No.	Compound Name	Binding Energy (Kcal/Mol)
1.	Choline	-3.99 Kcal/Mol
2.	Harmine	-3.71 Kcal/Mol
3.	Cuminaldehyde	-4.00 Kcal/Mol
4.	Cinnamic Acid	-5.20 Kcal/Mol
5.	Curcumrin	-2.42 Kcal/Mol
6.	Ursolic Acid	-3.43 Kcal/Mol
7.	Chlorogenic Acid	-4.20 Kcal/Mol
8.	Assafoetidnol B	-3.82 Kcal/Mol
9.	Glucobrassicin	-3.65 Kcal/Mol
10.	Linamarin	-2.32 Kcal/Mol
11.	Luteolin	-4.19 Kcal/Mol
12.	Amprenavir	-0.84 Kcal/Mol
13.	Acyclovir	-3.24 Kcal/Mol
14.	Umifenovir	-2.30 Kcal/Mol
15.	Combivir	-1.23 Kcal/Mol
16.	Tamiflu	-3.14 Kcal/Mol
17.	Zanamivir	-2.00 Kcal/Mol
18.	Thymoquinone	-4.71 Kcal/Mol
19.	Cidofovir	-1.78 Kcal/Mol
20.	Peramivir	-3.84 Kcal/Mol
21.	Andrographolide(Kalmegh)	-4.63 Kcal/Mol
22.	Greentea	-3.25 Kcal/Mol
23.	Citral	-2.82 Kcal/Mol
24.	Hydroxychloroquine	-2.70 Kcal/Mol
25.	Linalool	+1.49 Kcal/Mol
26.	Nicotine	-1.27 Kcal/Mol
27.	Chloroquine	-2.52 Kcal/Mol
28.	Allium	+ 0.58 Kcal/Mol

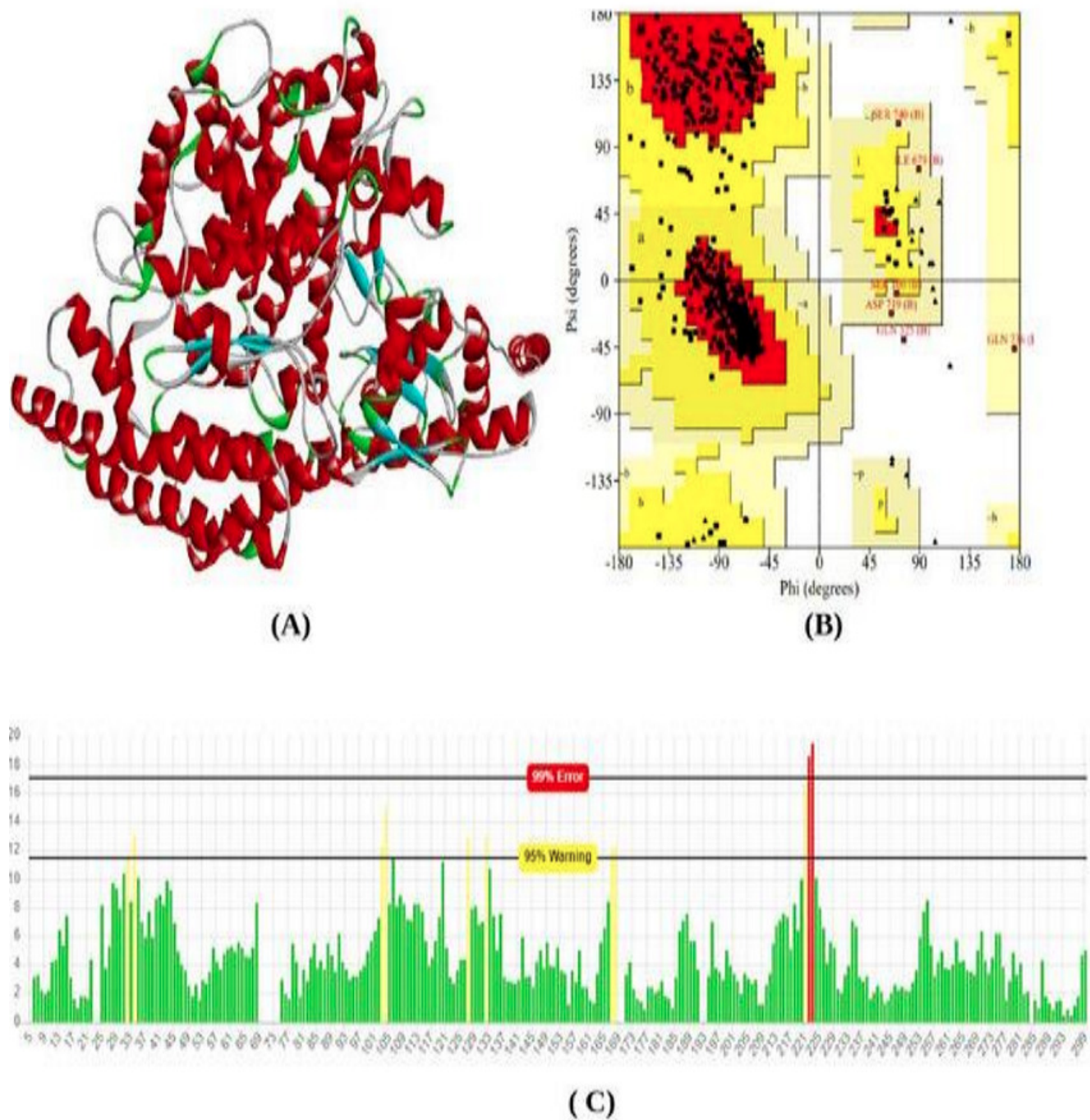
Best_Five_Docking_Result.

1   **Table5.** Result of top five best docking score with H-bond interaction.

S.No.	Compound Name	Binding Energy (Kcal/Mol)	H-Bond Interaction
1.	**Cinnamic acid**	-5.20 kcal/Mol	ALA-264,LYS-481, LEU-503
2.	**Thymoquinone**	-4.71 kcal/Mol	LEU-103, TRP-165, ARG-169
3.	**Andrographolide(Kalmegh)**	-4.63 Kcal/Mol	ARG-273, TYR-127, ASN-277, ASN-277
4.	**Cholengenic acid**	-4.20 kcal/ Mol	ARG-273, ARG-273
5.	**Cuminaldehyde**	-4.00 Kcal/Mol	TRP-165

Figure 1

Fig1._Protein_modeled_structure_.jpg

(A)   (B)

(C)
Figure 2

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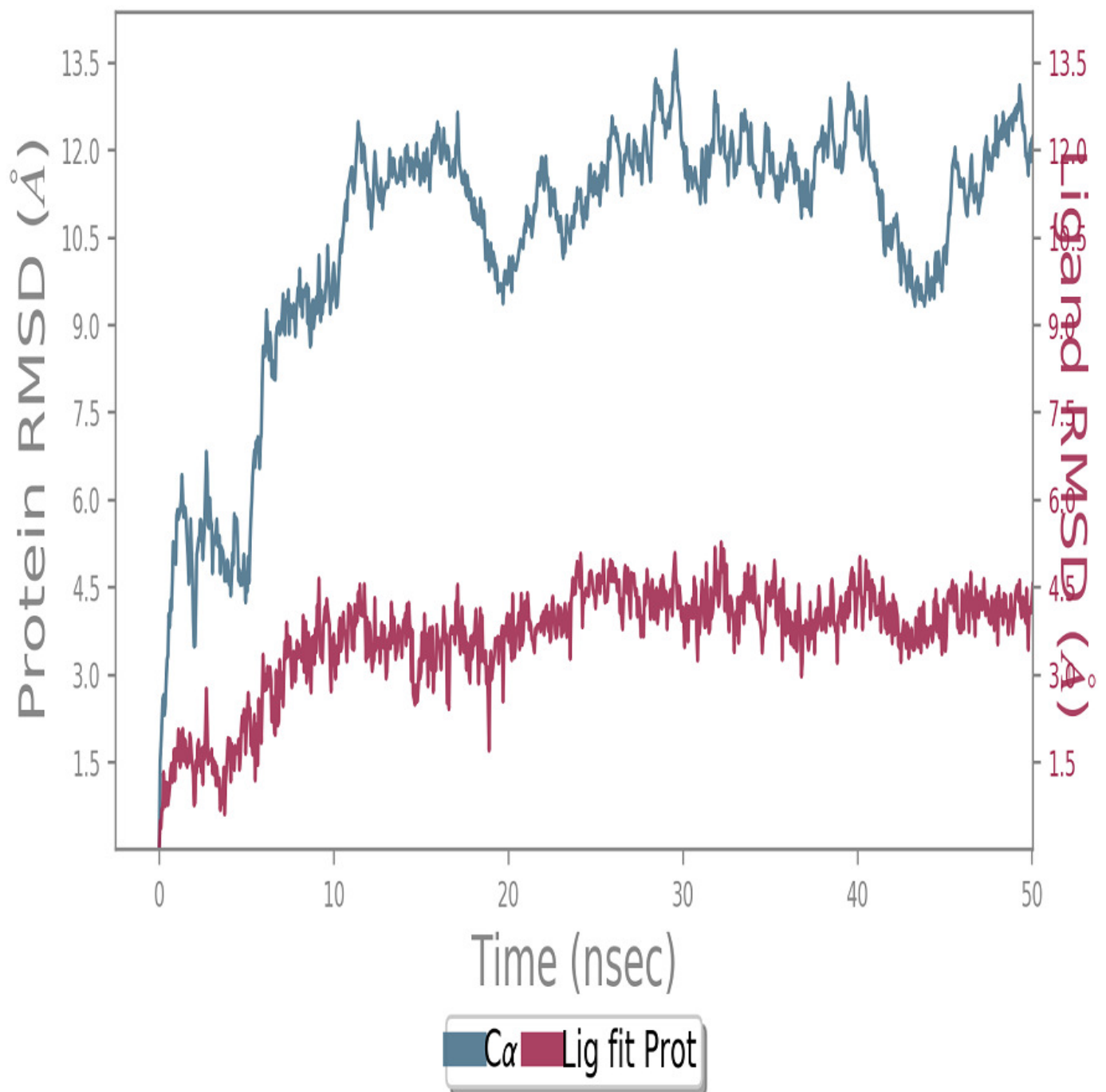
Figure 3

Fig2.Molecular_interaction_study.jpg
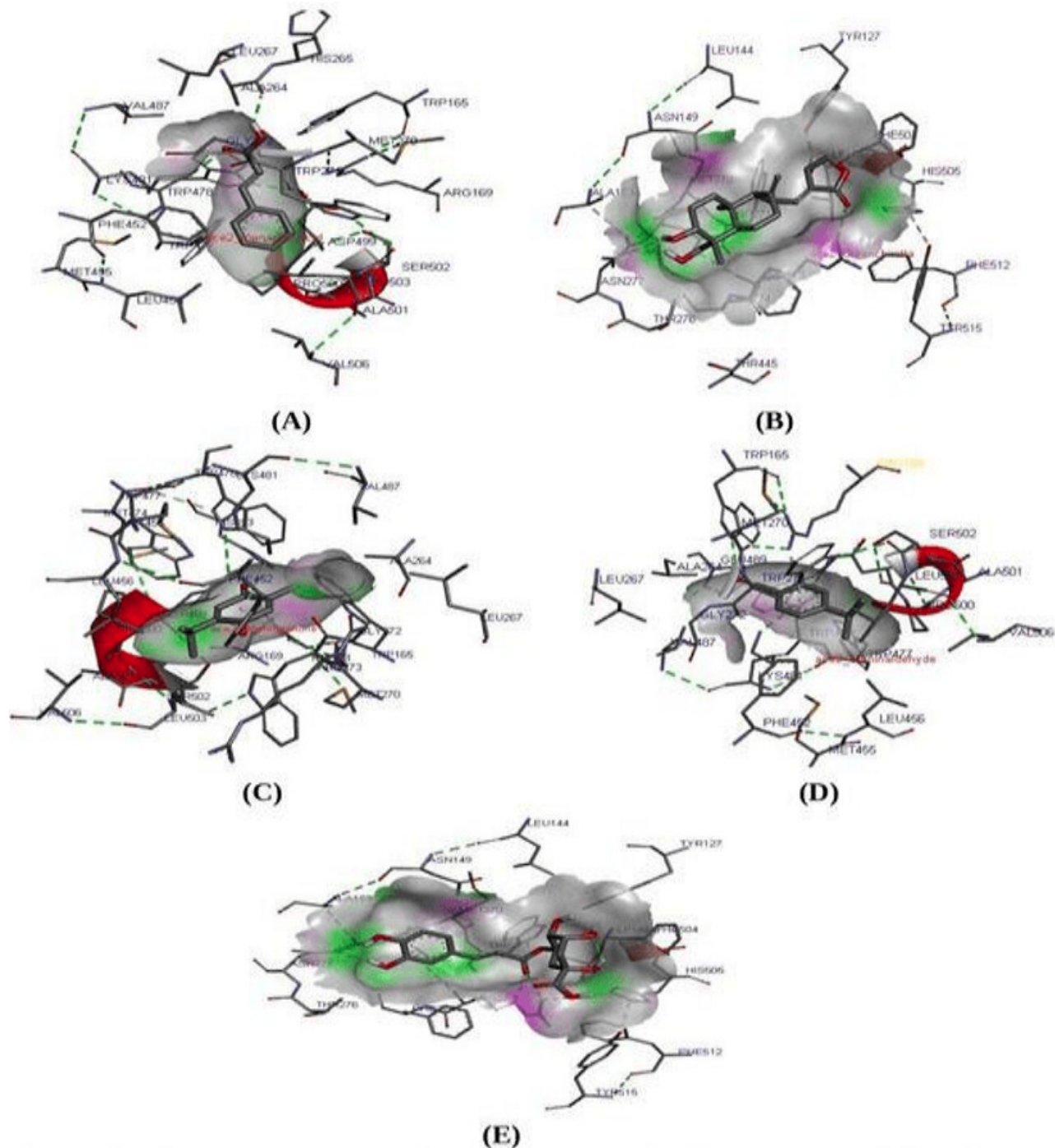
Figure 4

Fig4._Molecular_dynamic_simulation_study_of_ACE2-Thymoquinone.png
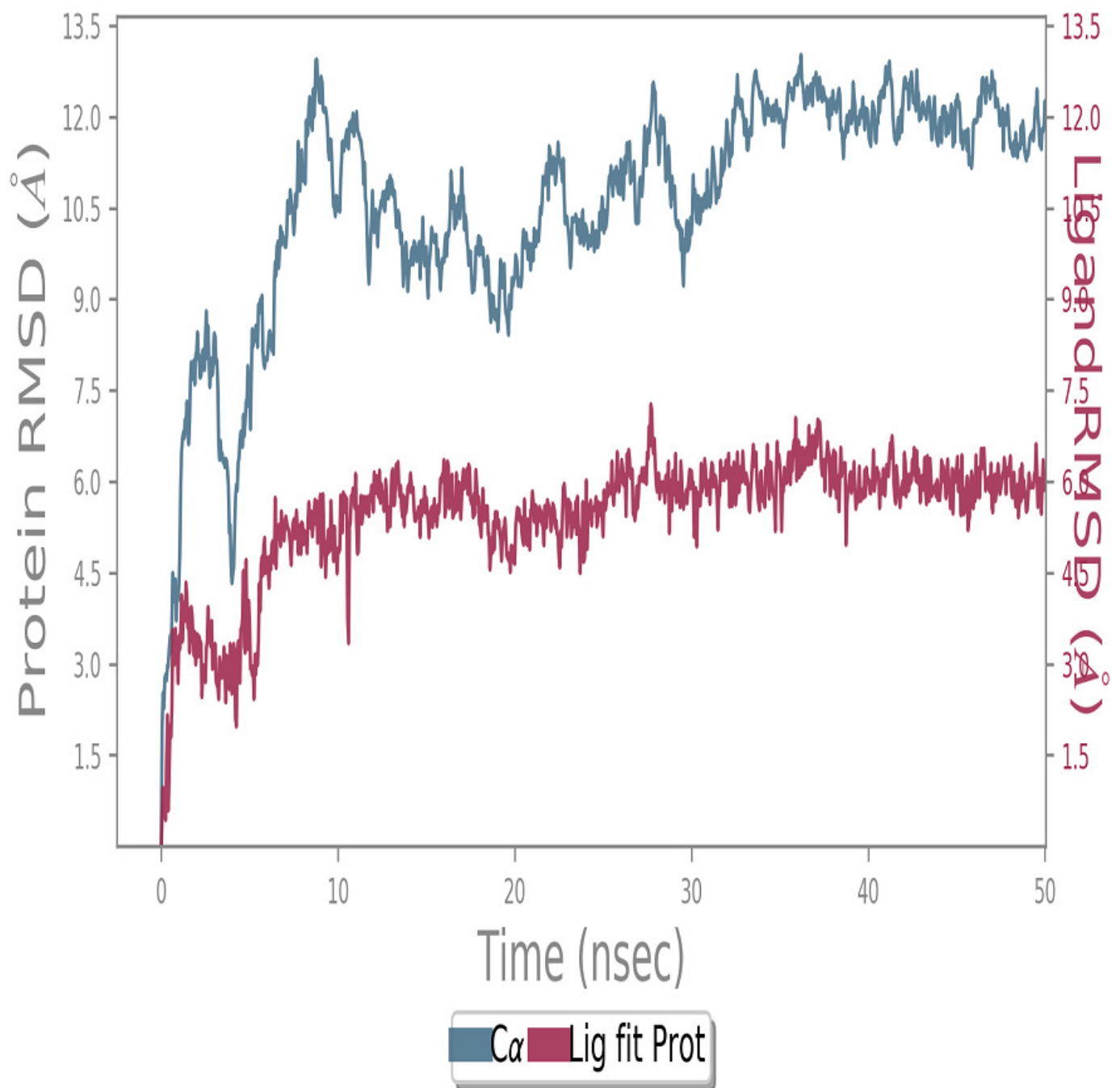
Figure 5

Molecular_dynamic_simulation_study_of_ACE2-Andrographolide_.png