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Effect of Vellai Erukkan Samula Parpam in the prevention and Management of SARS-CoV-2 and COVID-19 by inhibiting ACE2 Receptor

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Abstract

In December 2019, a cluster of Pneumonia cases, caused by a newly identified β-coronavirus, occurred in Wuhan, China. This Coronavirus was initially named as the 2019-novel Coronavirus (2019-nCoV) on 12 January 2020 by World Health Organization (WHO). **Objective:** In this study we execute a rational screen to identify Traditional Siddha medicine (Calatropis procera) in treating viral respiratory infections and also contain compounds that might directly inhibit 2019 novel coronavirus (2019-nCoV). **Methods:** Docking calculations were carried out for retrieved phytocomponents against target protein ACE-2. Essential hydrogen atoms, Kollman united atom type charges, and solvation parameters were added with the aid of AutoDock tools (Morris, Goodsell et al., 1998). **Results:** Binding of phytocomponents with the core amino acids (**31 LYS and 353 LYS**) of the target by forming hydrogen bond will hinder the function of the target Angiotensin-converting enzyme 2 (ACE2) receptors - **PDB- 2AJF** being recognized as binding site for novel corona virus for its pathogenesis essential for host-viral interaction.Total of **8** bioactive lead compounds were retrieved from the herbs present in the formulation *Vellai Erukkan Samula Parpam*. From reported data of the herb, the lead Rutin and Beta-Sitosterol possess **100% binding efficacy** by interacting with both the core target amino acids (**31 LYS and 353 LYS**) present on the target. Followed by this other phytocompounds such as quercetin 3-O-galactoside, Calotropagenin, Calotropin, Uscharidin and Coroglaucigenin possess **50% affinity** by ninding with one tagrt amino acid (**353 LYS**). **Conclusion:** Based on the results of the computational analysis it was concluded that the bio-active compound's such as Rutin, Beta-Sitosterol, quercetin 3-O-galactoside, Calotropagenin, Calotropin, Uscharidin and Coroglaucigenin present in the herbs of the formulation *Vellai Erukkan Samula Parpam* revels significant binding against the target protein thereby it was concluded that these compounds may exerts promising inhibiting against ACE-2 receptor and hereby **halt the host-viral interface**.Pre-clinical&clinical study needs to be done to confirm the proposed efficacy of the Vellai Erukkan Samula Parpam in the prevention of the Novel Corona Virus.

Keywords: Vellai Erukkan Samula Parpam (*Calotropis procera*), Anti-Viral Herbs, Siddha Medicine, SARS-CoV-2 COVID-19, Angiotensin-converting enzyme 2(ACE2), *In-Silico* Molecular Docking analysis.

Introduction

This study aims to assess the Indian Traditional Siddha herbal plant (*Calotropis procera*) in the pursuit of potential COVID-19 inhibitors using *in Silico* approaches. In December 2019, a cluster of Pneumonia cases, caused by a newly identified β-coronavirus, occurred in Wuhan, China. This Coronavirus was initially named as the 2019-novel Coronavirus (2019-nCoV) on 12 January 2020 by World Health Organization (WHO). WHO officially named the disease as Coronavirus disease 2019 (Covid-19) and Coronavirus Study Group (CSG) of the International Committee proposed to name the new Coronavirus SARS-CoV-2 both issued on 11 February 2020.

The Chinese scientists rapidly isolated a SARS-CoV-2 from a patient within a short time on 7 January 2020 and came out to genome sequencing of the SARS-CoV-2. As of 1March 2020, a total of 79,968 cases of Covid-19 have been confirmed in mainland China including 2873 deaths. Studies estimated the basic reproduction number (R0) of SARS-CoV-2 to be around 2.2 or even more (range from 1.4 to 6.5) and familial clusters of Pneumonia outbreaks add to evidence of the epidemic Covid-19 steadily growing by human-to-human transmission. [1]

Clinical manifestations and staging of Covid – 19 [3-4]

Chinese CDC report divided the clinical manifestations of the disease based on their severity

Mild disease:

Non-pneumonia and mild pneumonia.
(This occurred in 81% of cases)

Severe disease:

Dyspnea, respiratory frequency ≥30/min, blood oxygen saturation (SpO2) s 93%, and or lung infiltrates > 50% within 24 to 48 hours this occurred in 14% of cases)

Critical disease:

Respiratory failure, septic shock, and or multiple organ dysfunction (MOD) or failure (MOF). (This occurred in 5% of cases). [3-4]

A Siddha Perspective of Covid-19[5-6]

The Siddha system of medicine is mainly practised in Southern part of India. It is one of the earliest traditional system in the world which treats not only the body but also mind and the soul. The word Siddha has its origin in the tamil word Siddhu which means **"perfection"** or **"heavenly bliss".** Siddha medicine classifies disease and disorders into **4448** types. In Siddha literature, YUGI VAITHIYA CHINTHAMANI about **64** types of SURAM (**Fever**) are described. Among them **SANIPATHA SURAM (ABINIYASA SANNI**) is one which may be correlated to SARS-COV-2 infection and COVID-19 disease. Siddha encloses a unique technique by elaborating the disease by Envagai thervu (Diagnostic technique), Noi varum vazhi (Etiological factors), Mukkutra verupaadu (Deranged humors), Mukkuri gunangal (Pathological symptoms). [5-6]

Novel Corona virus is making its Worldwide propagation in a very fast phase. It is now essential to discover the drugs that are useful in the prevention and management of SARS CoV-2 and Covid-19.Many traditional Herbs and Poly Herbal synergistic formulations are useful in the prophylaxis of various types of Viruses. In Siddha system of medicine, there are various medicines used for Anti-Viral therapies.

To prove safety and efficacy of a traditional medicine, **Reverse Pharmacology Method** is recognized globally. Reverse pharmacology is confirming the safety and efficacy of a medicine which is already in clinical practice by going back in the steps of pharmacological screening and drug development. The ultimate aim of the Reverse pharmacological research is to find the mechanism of action by a drug against a disease. For ***Vellai Erukkan Samula Parpam*** (In classical Siddha literature - *The Pharmacopoeia of Siddha Research Medicines– Chapter-1, Pg.no75, NO93. Dr.M. Shanmugavelu, Dr.G.D.Naidu. Published Sri G.D.Naidu, printed IL WA Press, Coimbatore-18)*[2].In this study, we have done the **In-Silico Molecular Docking Analysis** of the Bio-active compounds found in the aqueous extract of ***Vellai Erukkan Samula Parpam*** against the **ACE2 Enzyme receptor**.Which is the route of entry in the pathogenesis of Noval Corona Virus. Pre-clinical& clinical study needs to be done to confirm the proposed efficacy of the Vellai Erukkan Samula Parpam in the prevention of the Novel Corona Virus.

Objective:

Binding of phytocomponents with the core amino acids (31 LYS and 353 LYS) of the target by forming hydrogen bond will hinder the function of the target Angiotensin-converting enzyme 2 (ACE2) receptors - **PDB- 2AJF** being recognized as binding site for novel corona virus for its pathogenesis essential for host-viral interaction. Thereby phytocomponents which inhibit the target ACE-2 may act as a potential therapeutic agent for management of COVID-19 and related symptoms.

PDB	Name of the Target
2AJF	Angiotensin-converting enzyme 2 (ACE2) receptor

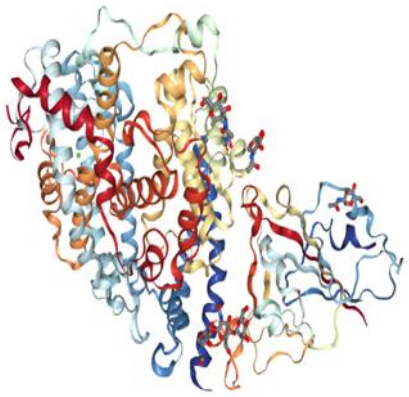
Materials and Methods

Docking calculations were carried out for retrieved phytocomponents against target protein ACE-2. Essential hydrogen atoms, Kollman united atom type charges, and solvation parameters were added with the aid of AutoDock tools (Morris, Goodsell et al., 1998). Affinity (grid) maps of ×× Å grid points and 0.375 Å spacing were generated using the Autogrid program (Morris, Goodsell et al., 1998). AutoDock parameter set- and distance-dependent dielectric functions were used in the calculation of the van der Waals and the electrostatic terms, respectively. Docking simulations were performed using the Lamarckian genetic algorithm (LGA) and the Solis & Wets local search method (Solis and Wets, 1981). Initial position, orientation, and torsions of the ligand molecules were set randomly. All rotatable torsions were released during docking. Each docking experiment was derived from 2 different runs that were set to terminate after a maximum of 250000 energy evaluations. The population size was set to 150. During the search, a translational step of 0.2 Å, and quaternion and torsion steps of 5 were applied.

List of Phytocomponents Selected for docking

1. Rutin[11]
2. Quercetin 3-O-galactoside [11]
3. Calotropagenin, [11]
4. Calotropin[11]
5. Uscharidin[11]
6. Coroglaucigenin [11]
7. β-sitosterol[12]
8. R-limonene[13]

3D- Structure of Angiotensin-converting enzyme 2 (ACE2) receptor- PDB 2AJF



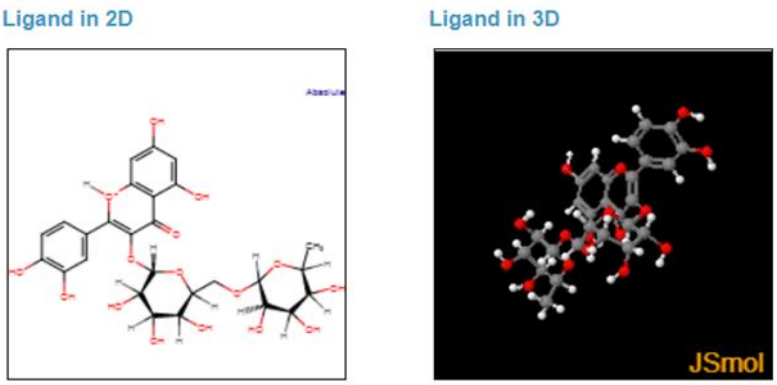
Receptor structure

Crystalline structure of the target protein Angiotensin-converting enzyme 2 (ACE2) receptor- PDB 2AJF was retrieved from protein data bank and protein clean-up process was done and essential missing hydrogen atom were being added. Different orientation of the lead molecules with respect to the target protein was evaluated by Autodock program and the best dock pose was selected based on the interaction study analysis.

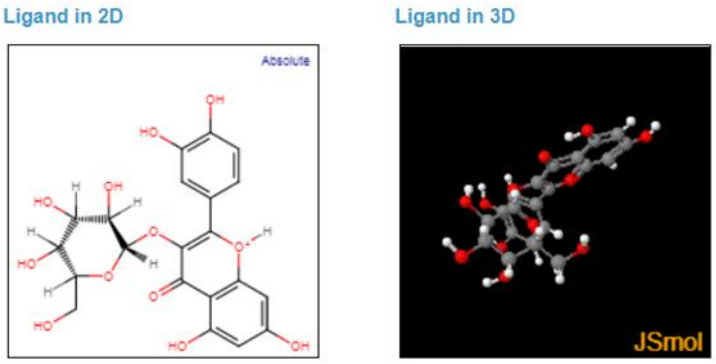
[illegible] [2]	**CALOTROPIS PROCERA**	**WHOLE PLANT**

2D and 3D Structure of Selected Ligands

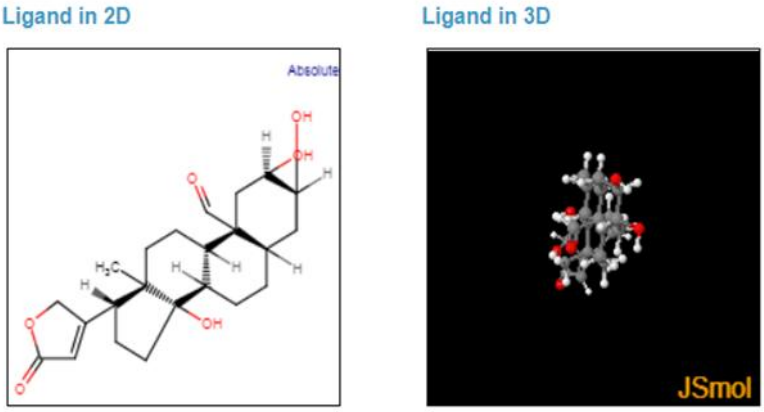
Rutin



Quercetin 3-O-galactoside

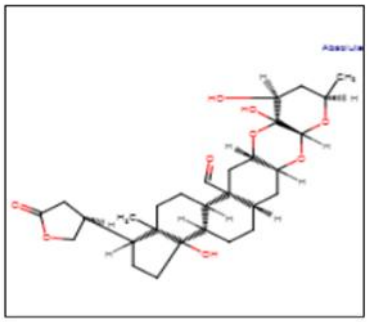


Calotropagenin



Calotropin

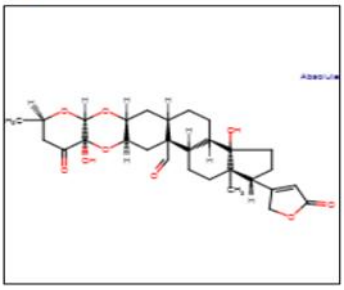
Ligand in 2D



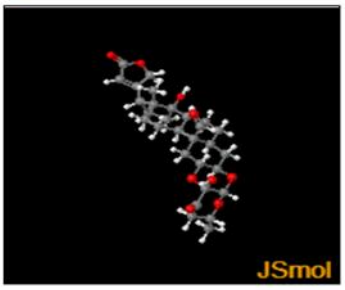
Ligand in 3D

Uscharidin

Ligand in 2D

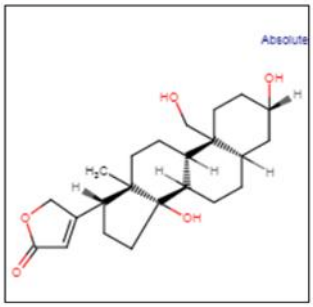


Ligand in 3D



Coroglaucigenin

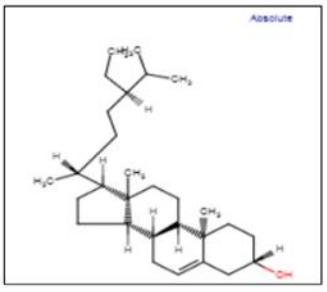
Ligand in 2D



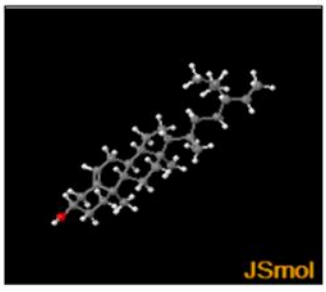
Ligand in 3D

β-sitosterol

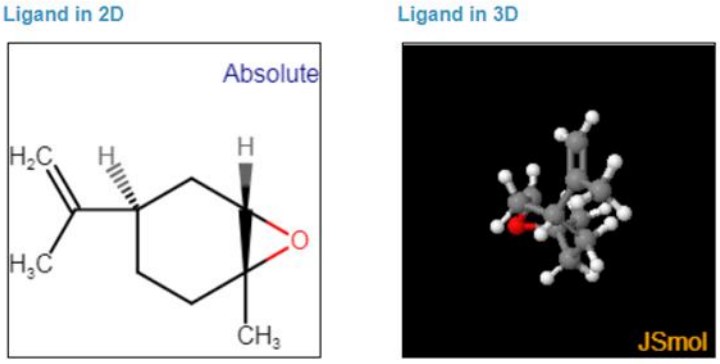
Ligand in 2D



Ligand in 3D



R-limonene



Ligand Properties of the Compounds Selected for Docking Analysis

Compound	Molar weight g/mol	Molecular Formula	H Bond Donor	H Bond Acceptor	Rotatable bonds
Rutin	610.5 g/mol	$C_{27}H_{30}O_{16}$	10	16	6
Quercetin 3-O-galactoside	626.5 g/mol	$C_{27}H_{30}O_{17}$	11	17	7
Calotropagenin	404.5 g/mol	$C_{23}H_{32}O_6$	3	6	2
Calotropin	548.6 g/mol	$C_{29}H_{40}O_{10}$	4	10	2
Uscharidin	530.6 g/mol	$C_{29}H_{38}O_9$	2	9	2
Coroglaucigenin	390.5 g/mol	$C_{23}H_{34}O_5$	3	5	2
β-sitosterol	414.7g/mol	$C_{29}H_{50}O$	1	1	6
Limonene	136.23 g/mol	C10H16	0	0	1

Summary of the molecular docking studies of compounds against Angiotensin-converting enzyme 2 (ACE2) receptor- PDB 2AJF

Compounds	Binding Free energy Kcal/mol	Inhibition constant Ki µM (*mM)(**nM)	Electrostatic energy Kcal/mol	Intermolecular energy Kcal/mol	Total Interaction Surface
Rutin	-2.39	17.59*	-0.23	-5.94	596.23
Quercetin 3-O-galactoside	-4.72	345.88	-0.19	-5.66	822.11
Calotropagenin	-4.85	277.98	-0.24	-6.21	579.60
Calotropin	-4.43	570.08	-0.17	-5.96	554.90
Uscharidin	-5.45	101.21	-0.09	-6.28	750.34
Coroglaucigenin	-5.10	183.26	-0.12	-5.59	465.66
β-sitosterol	-6.05	37.05	-0.20	-7.76	634.45
R-limonene	-4.06	1.06*	-0.01	-4.36	440.79

Amino acid Residue Interaction of Lead against Angiotensin-converting enzyme 2 (ACE2) receptor- PDB 2AJF

Molecule	Interactions	Amino Acid Residue- Binding										
Rutin	2	27 THR	31 LYS	34 HIS	35 GLU	38 ASP	353 LYS					
Quercetin 3-O-galactoside	1	30 ASP	34 HIS	37 GLU	353 LYS	386 ALA	389 PRO	393 ARG				
Calotropagenin	1	30 ASP	33 ASN	34 HIS	37 GLU	353 LYS	389 PRO	390 PHE	393 ARG			
Calotropin	1	30 ASP	33 ASN	34 HIS	37 GLU	353 LYS	393 ARG					
Uscharidin	1	36 LYS	29 LEU	30 ASP	33 ASN	34 HIS	37 GLU	93 VAL	96 GLN	353 LYS	389 PRO	393 ARG
Coroglaucigenin	1	34 HIS	35 GLU	37 GLU	38 ASP	353 LYS						
β-sitosterol	2	27 THR	30 ASP	31 LYS	34 HIS	35 GLU	38 ASP	353 LYS				
R-limonene	0	37 GLU	40 PHE	350 ASP	386 ALA	390 PHE	393 ARG					

Results

Observation and Inference

Total of 8 bioactive lead compounds were retrieved from the herbs present in the formulation Vellai Erukkan Samula Parpam. From reported data of the herb, the lead Rutin and Beta-Sitosterol possess **100% binding efficacy** by interacting with both the core target amino acids (**31 LYS and 353 LYS**) present on the target. Followed by this other phytocompounds such as quercetin 3-O-galactoside, Calotropagenin, Calotropin, Uscharidin and Coroglaucigenin possess **50% affinity** by ninding with one tagrt amino acid (**353 LYS**). Further the compound limonene did not reveal any significant biding affinity with the core amino acid present on the target receptor ACE-2.

Binding of phytocomponents with the core amino acids (**31 LYS and 353 LYS**) of the target by forming hydrogen bond will hinder the function of the target Angiotensin-converting enzyme 2 (**ACE2)** receptors - **PDB- 2AJF** being recognized as binding site for novel corona virus for its pathogenesis essential for host-viral interaction. Thereby phytocomponents which inhibit the target ACE-2 may act as a potential therapeutic agent for management of COVID-19 and related symptoms.

Discussion

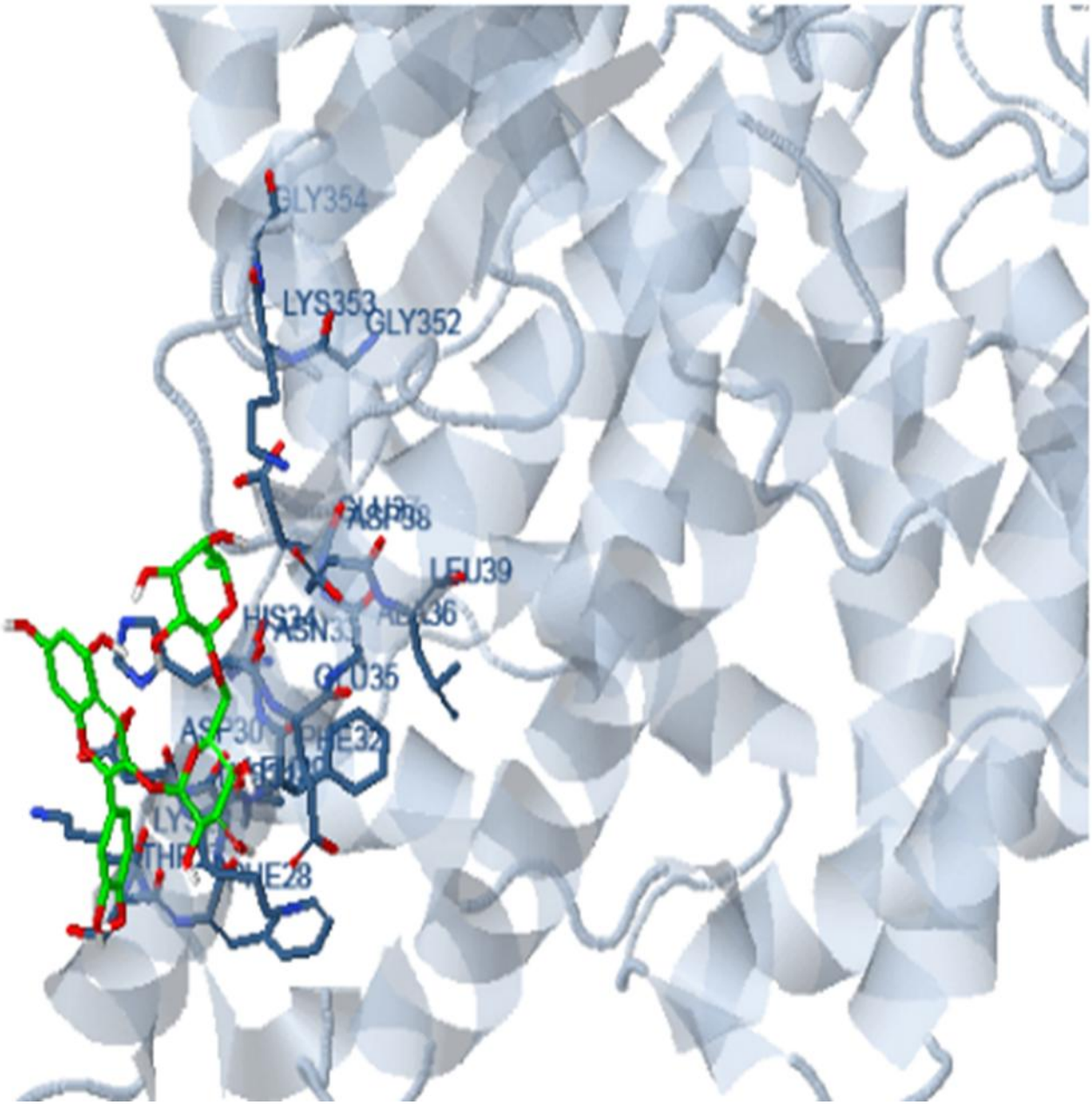
Crystalline structure of the target protein Angiotensin-converting enzyme 2 (ACE2) receptor- PDB 2AJF was retrieved from protein data bank and protein clean-up process was done and essential missing hydrogen atom were being added. Different orientation of the lead molecules with respect to the target protein was evaluated by Autodock program and the best dock pose was selected based on the interaction study analysis. The plant Calotropis procera have been researched more on toxicity, pharmacology evidence(Anti-Viral activity [18-19], Bronchodilator Activity, Anti-Histaminic activity[15], Immunomodulatory activity [16-17], Anti-Angiogenic activity, anti-diabetic, cardiovascular, Analgesic and Anti-pyritic activity, anti-inflammatory, antioxidant, Anti-Microbial activity, Anti-Cancer , Anti-Convulsant activity [14])from the published journal ,this Siddha trial drug will be ideology because it will be less economic in preparation and as raw source, this single drug will be suggestive for treating like acute , chronic respiratory illness , viral diseases, and also various respiratory symptoms like dyspnoea, shortness of breathing, Chest discomfort, Wheezing, breathlessness, cold , Cough with tenacious sputum and other respiratory diseases. a hypothesis is created in such a way that the efficacy of the VELLAI ERUKKAN SAMULA PARPAM will be a better solution for SARS-COV-2 infection and COVID-19 disease.

Conclusion

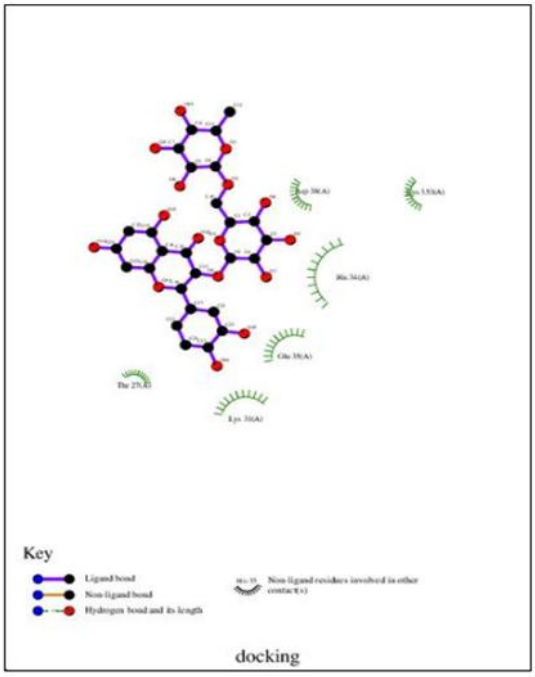
Based on the results of the computational analysis it was concluded that the bio-active compound's such as Rutin, Beta-Sitosterol, quercetin 3-O-galactoside, Calotropagenin, Calotropin, Uscharidin and Coroglaucigenin present in the herbs of the formulation ***Vellai Erukkan Samula Parpam*** revels significant binding against the target protein thereby it was concluded that these compounds may exerts promising inhibiting against ACE-2 receptor and hereby **halt** the **host-viral interface**. Pre-clinical & clinical study needs to be done to confirm the proposed efficacy of the *Vellai Erukkan Samula Parpam*is to validate the therapeutic efficacy and safety profile to administered for the SARS –V2-COVID-19 which has been more dreadful for the community and more challenging in treating the disease especially of severe respiratory illness which causes more severity.It can be helpful in improving our Indian economy through this medicine, I hope this drug will be safe and promising drug for SARS –V2-COVID-19.

Docking Pose

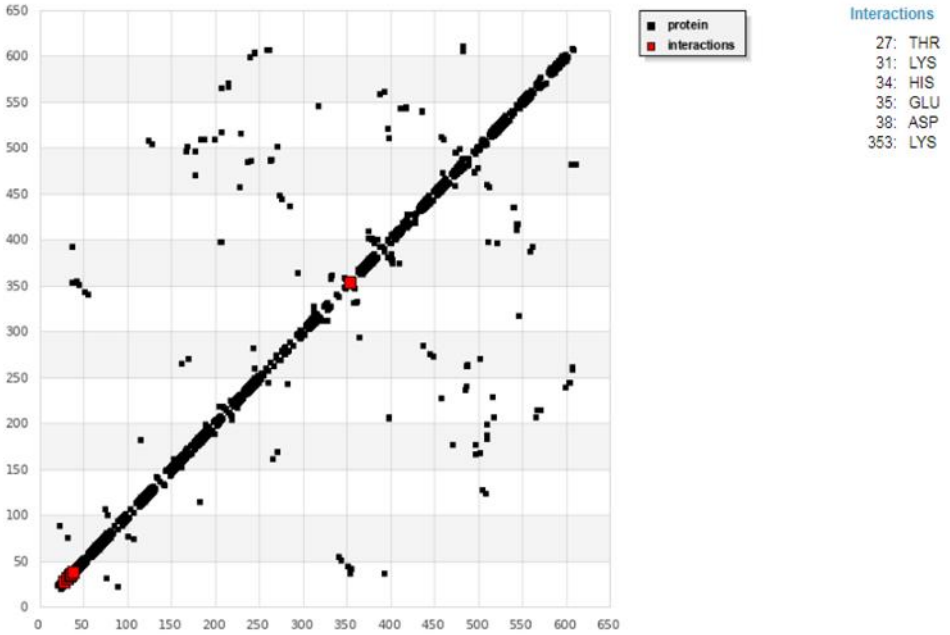
Rutin with Angiotensin-converting enzyme 2 (ACE2) receptor- PDB 2AJF



2D Interaction Plot

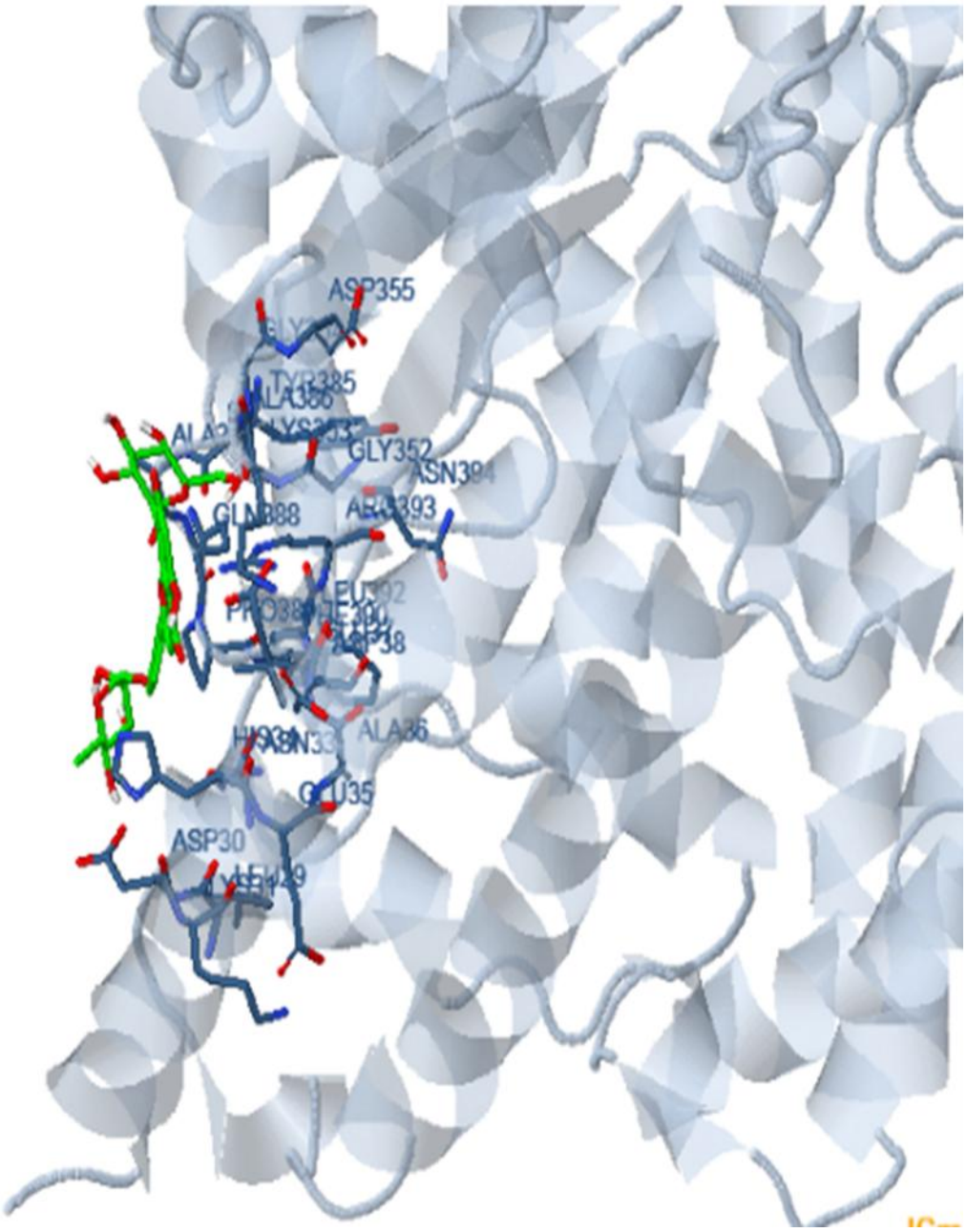


Hydrogen bond plotting with core amino acid Analysis

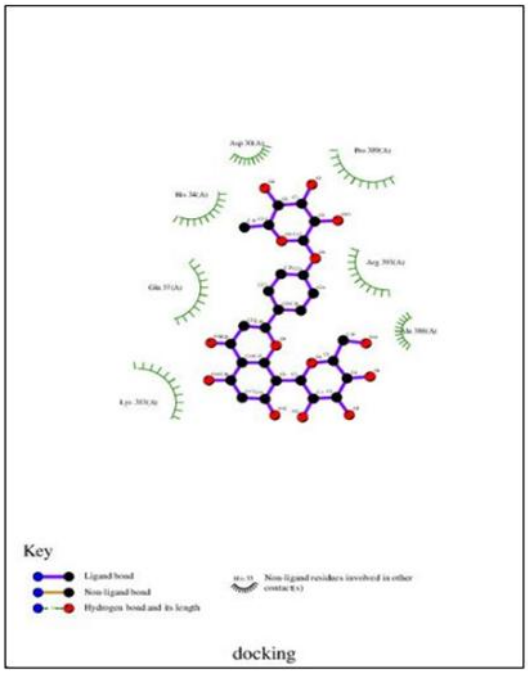


Docking Pose

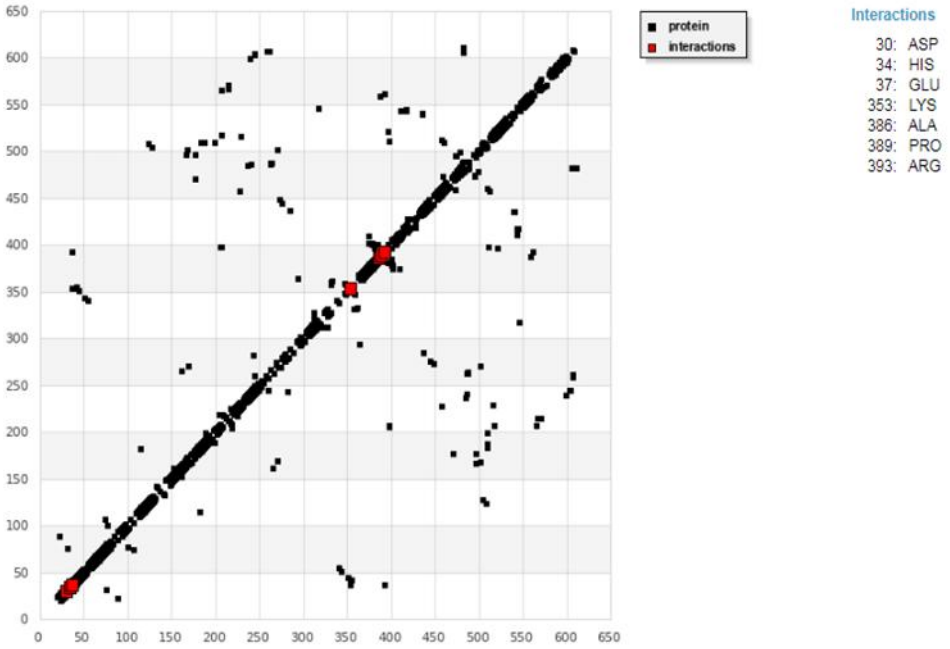
Quercetin 3-O-galactoside with Angiotensin-converting enzyme 2 (ACE2) receptor- PDB 2AJF



2D Interaction Plot

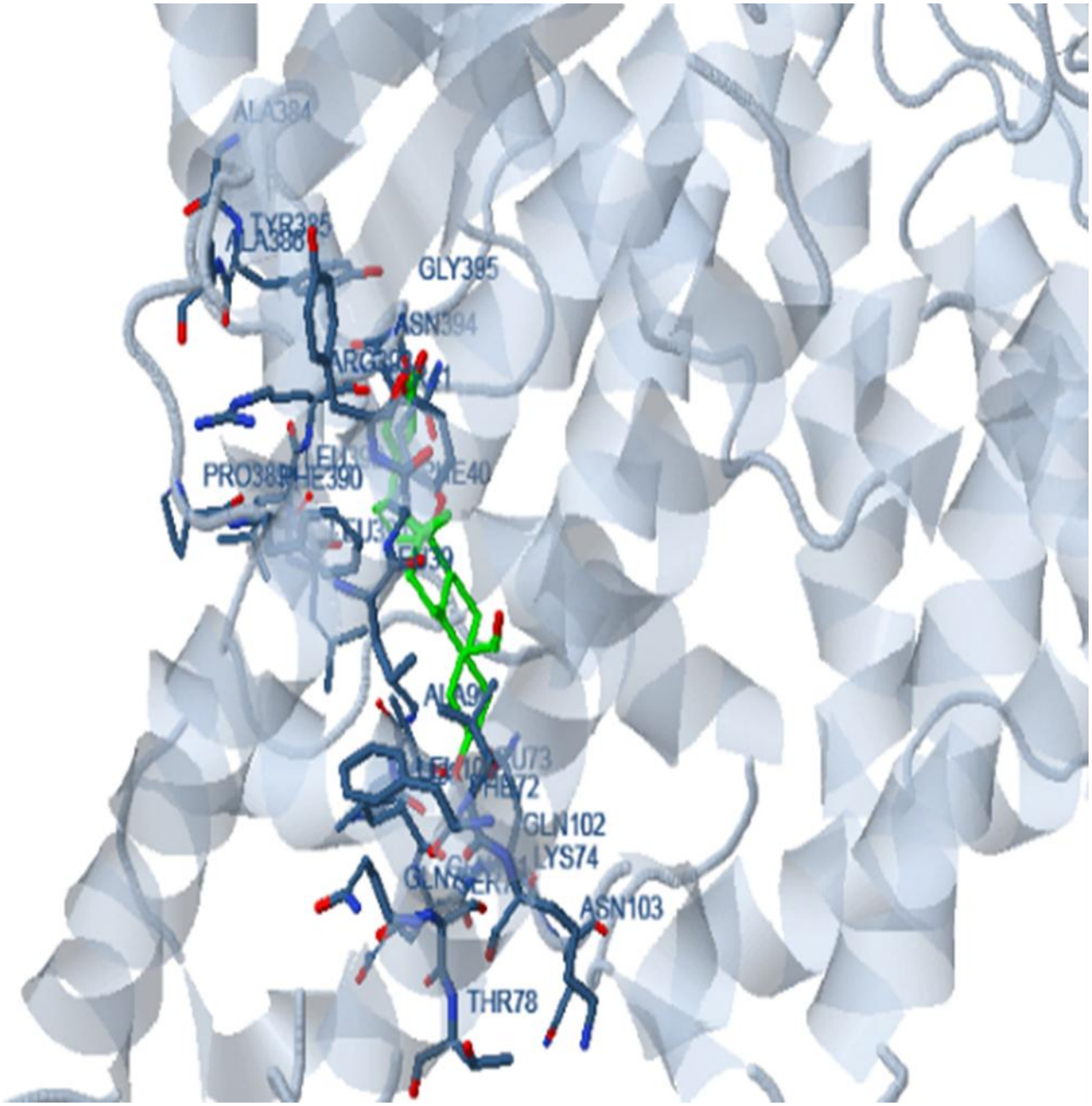


Hydrogen bond plotting with core amino acid Analysis

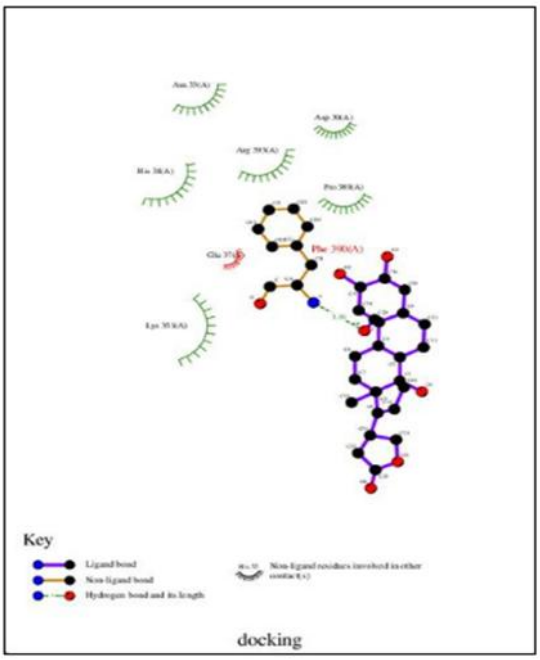


Docking Pose

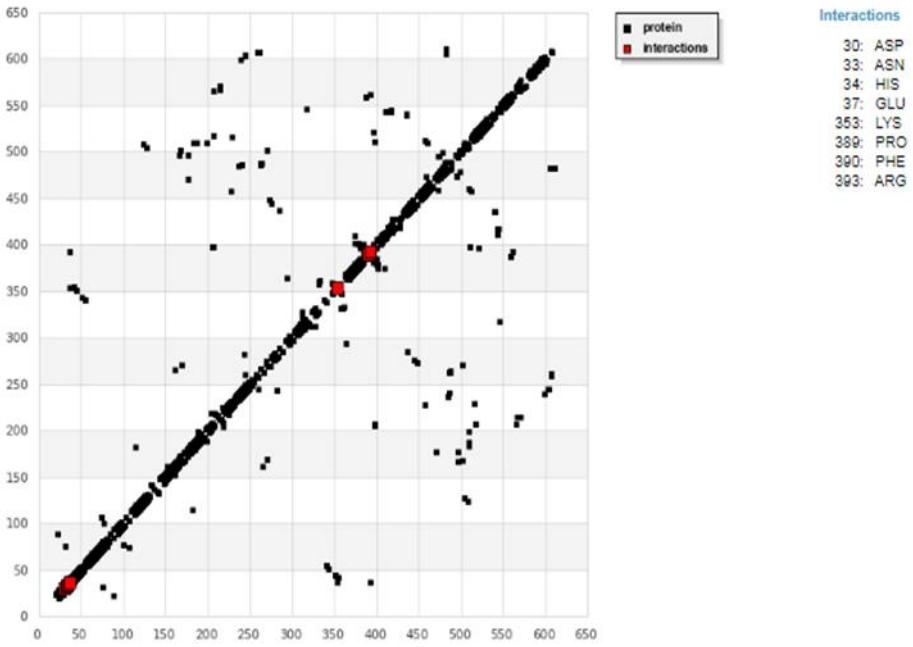
Calotropagenin with Angiotensin-converting enzyme 2 (ACE2) receptor- PDB 2AJF



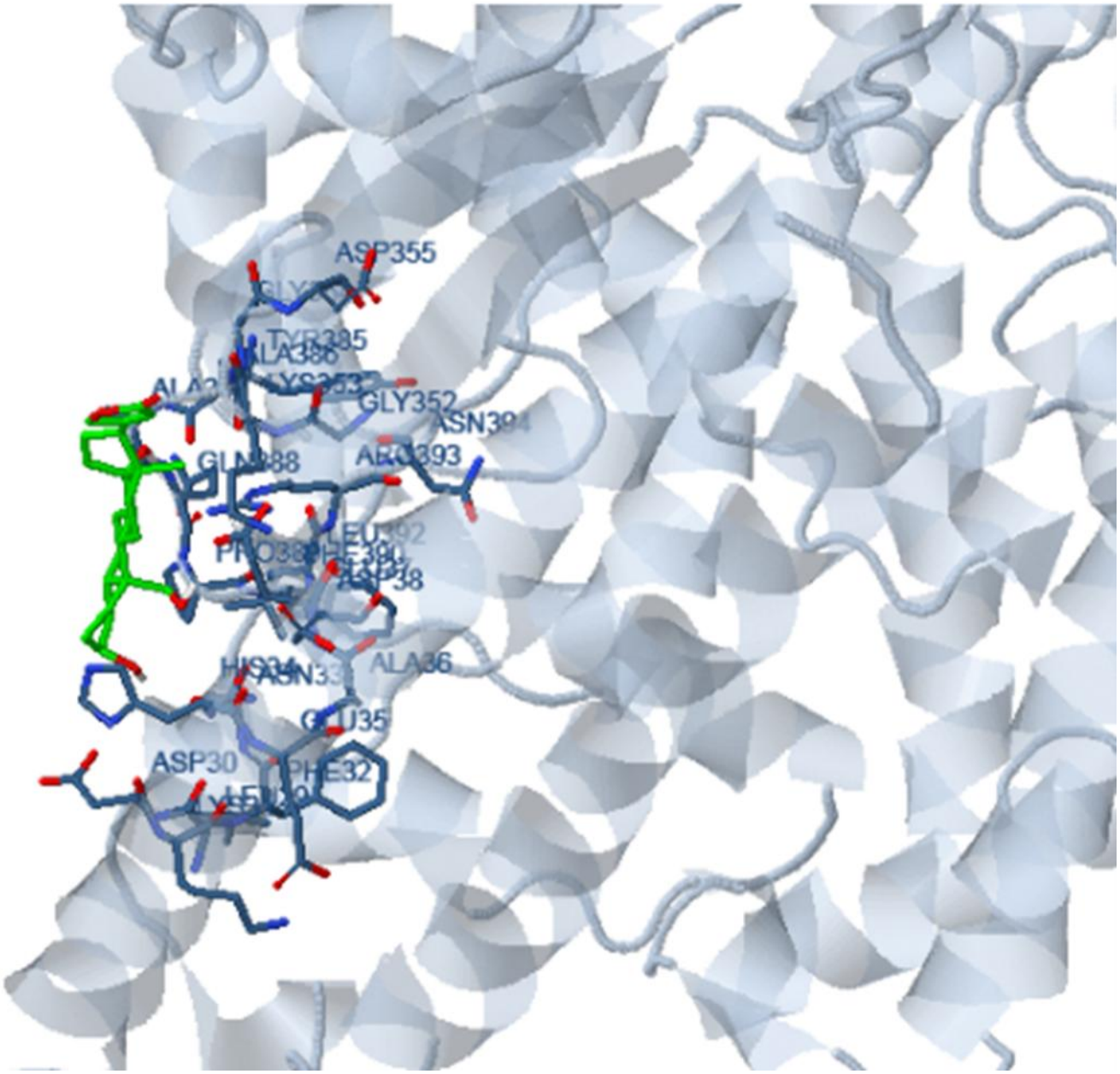
2D Interaction Plot



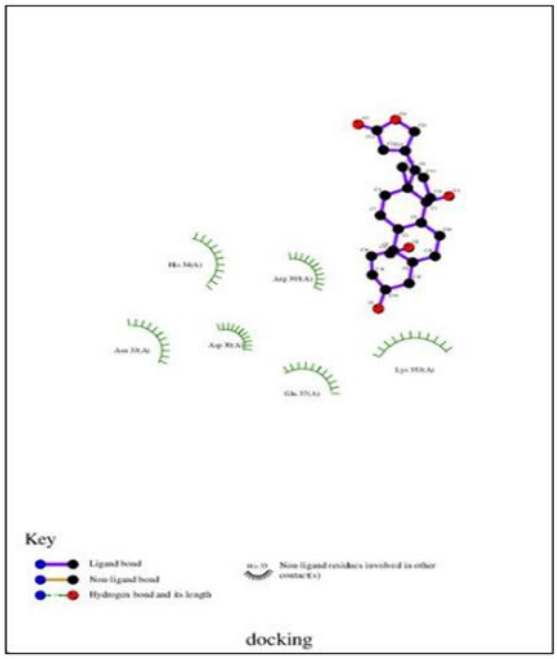
Hydrogen bond plotting with core amino acid Analysis



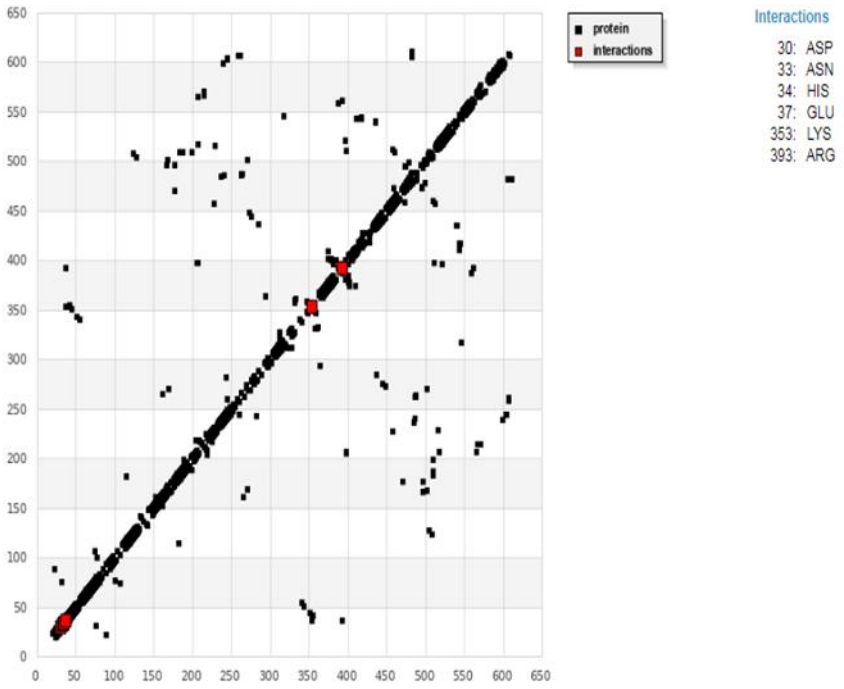
Docking Pose
Calotropin with Angiotensin-converting enzyme 2 (ACE2) receptor- PDB 2AJF



2D Interaction Plot

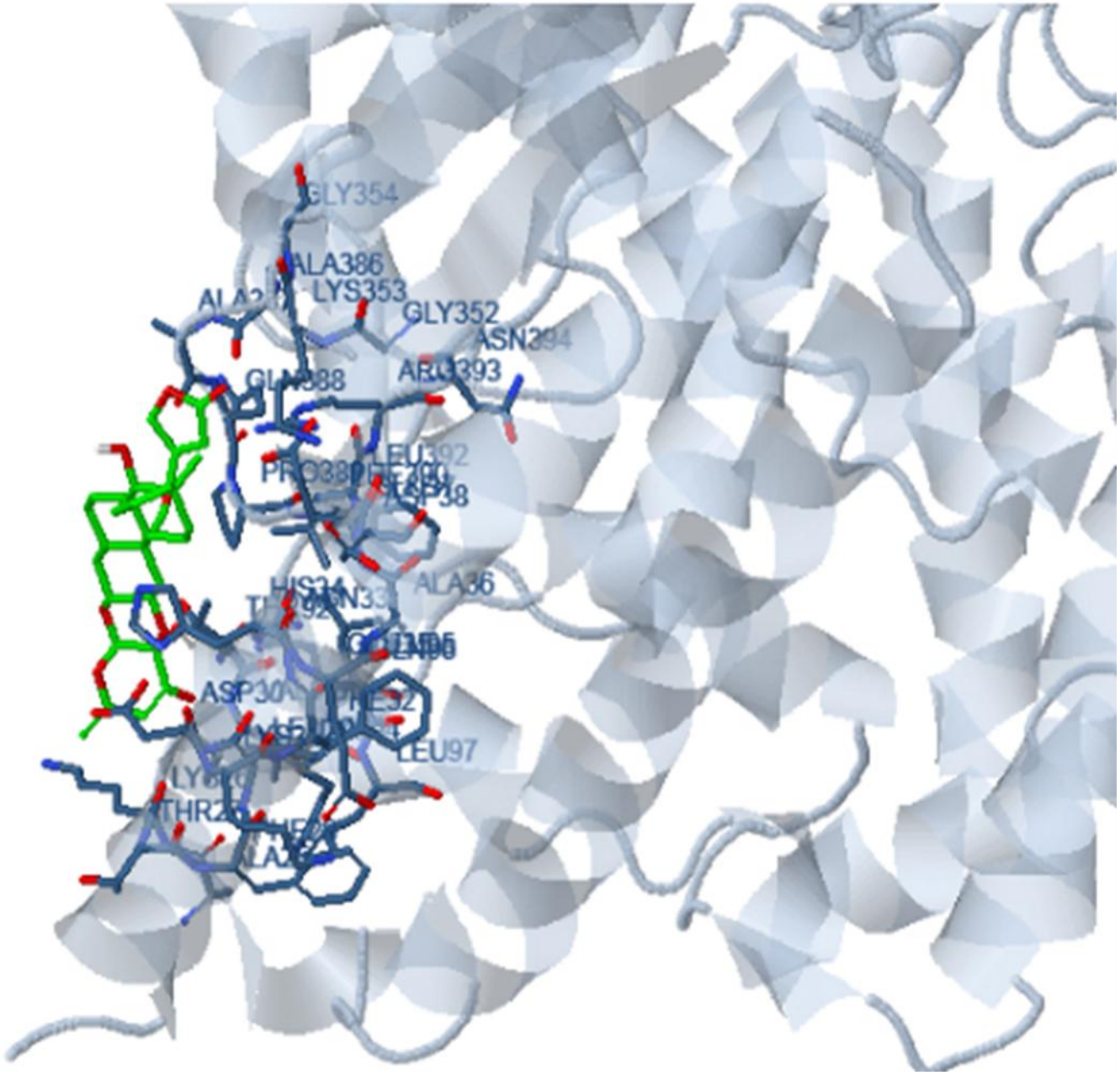


Hydrogen bond plotting with core amino acid Analysis

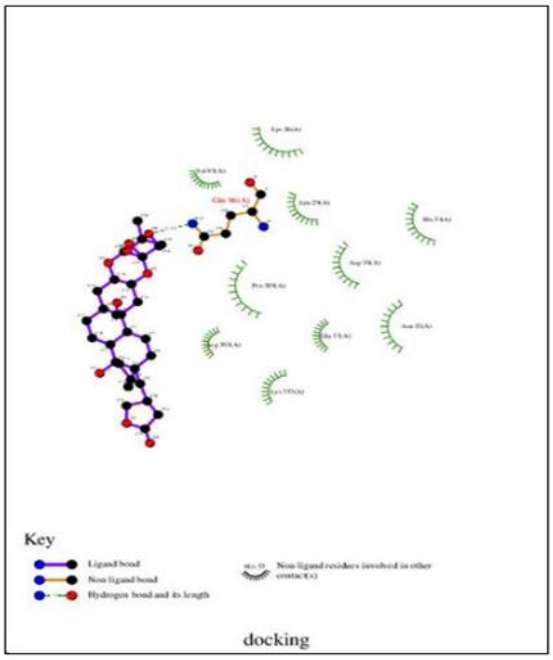


Docking Pose

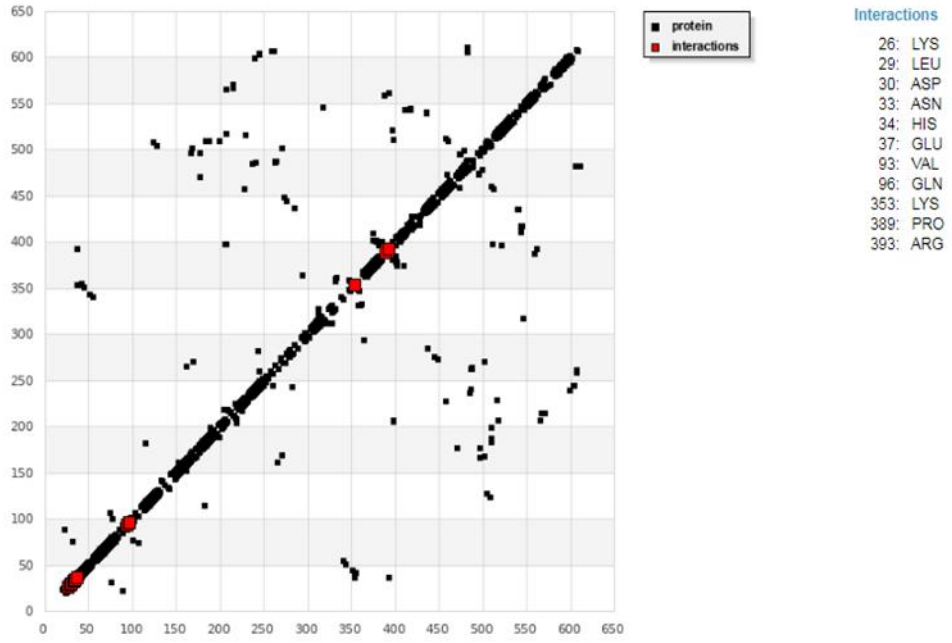
Uscharidin with Angiotensin-converting enzyme 2 (ACE2) receptor- PDB 2AJF



2D Interaction Plot

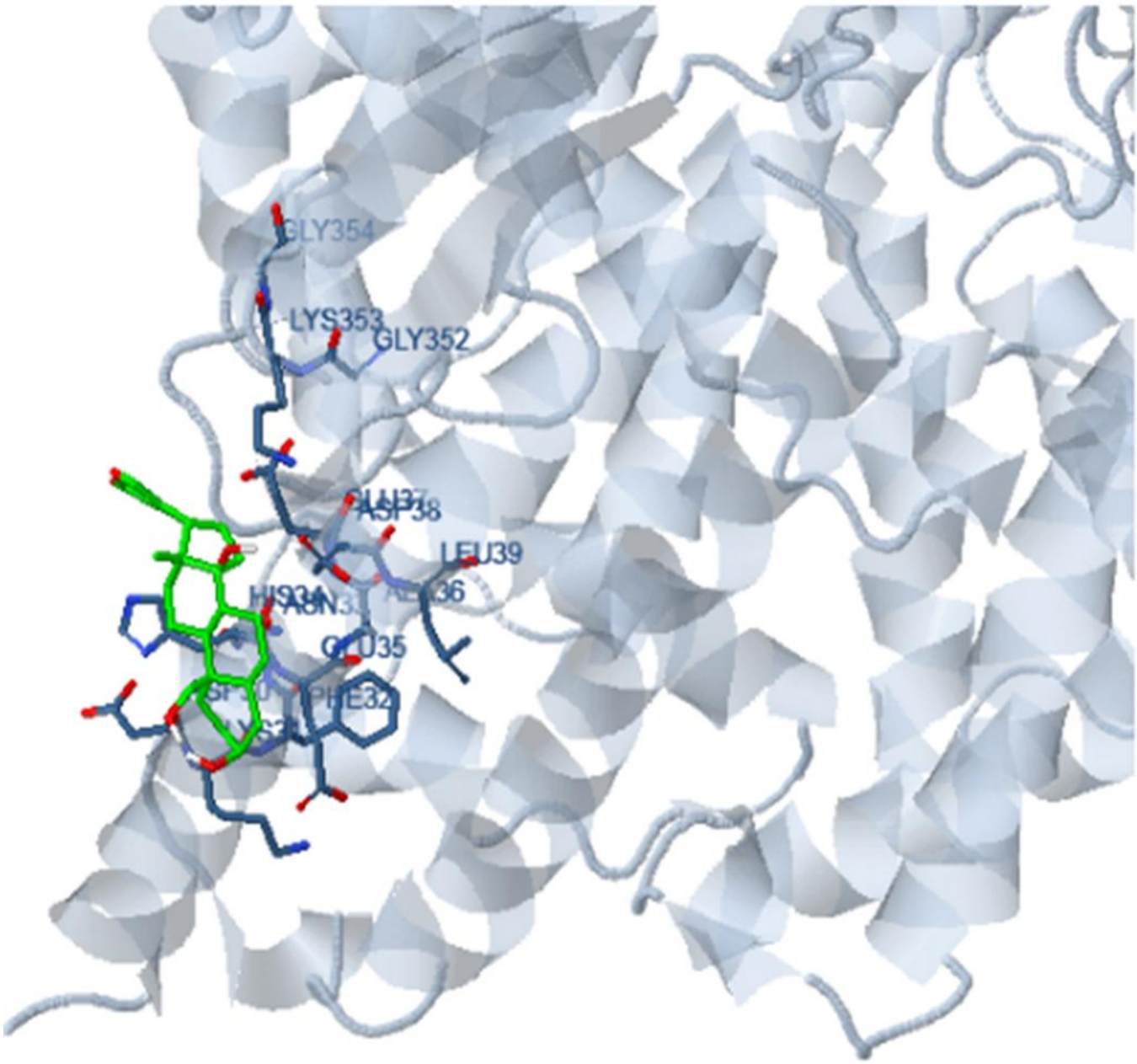


Hydrogen bond plotting with core amino acid Analysis

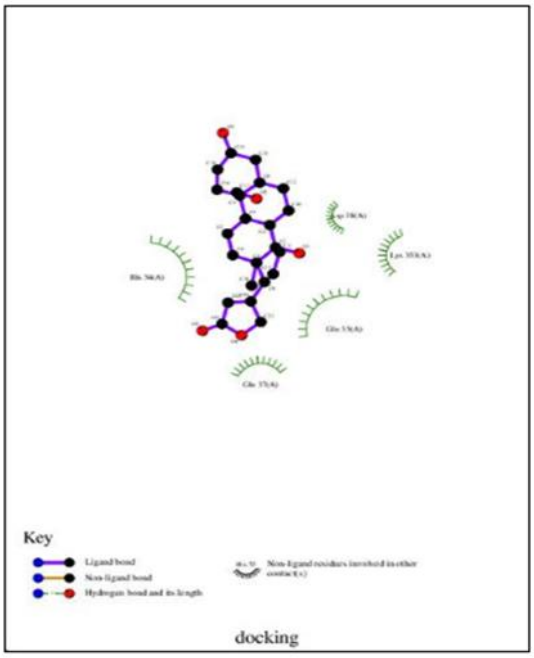


Docking Pose

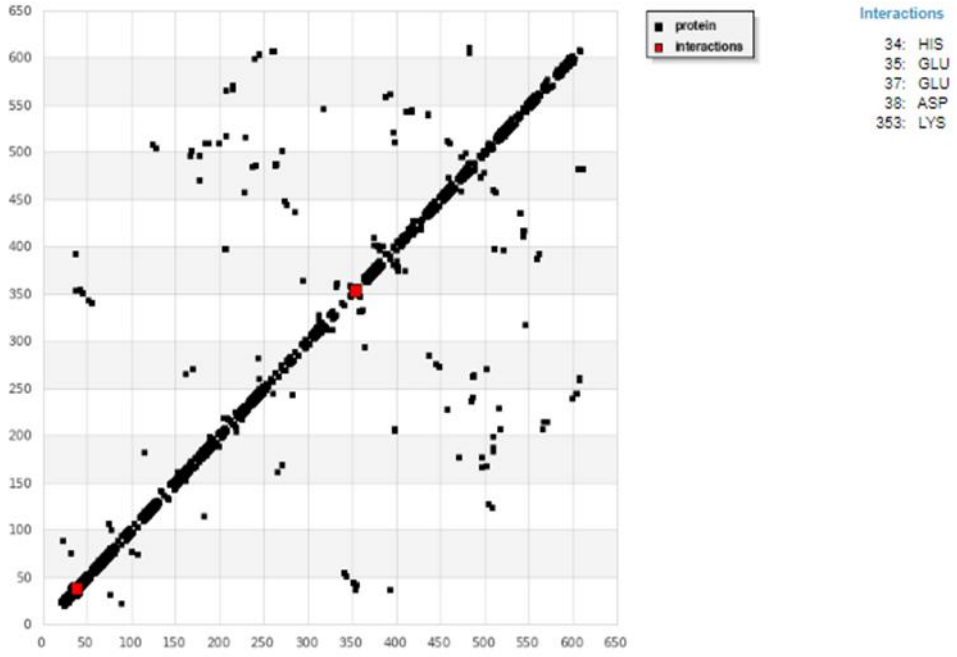
Coroglaucigenin with Angiotensin-converting enzyme 2 (ACE2) receptor- PDB 2AJF



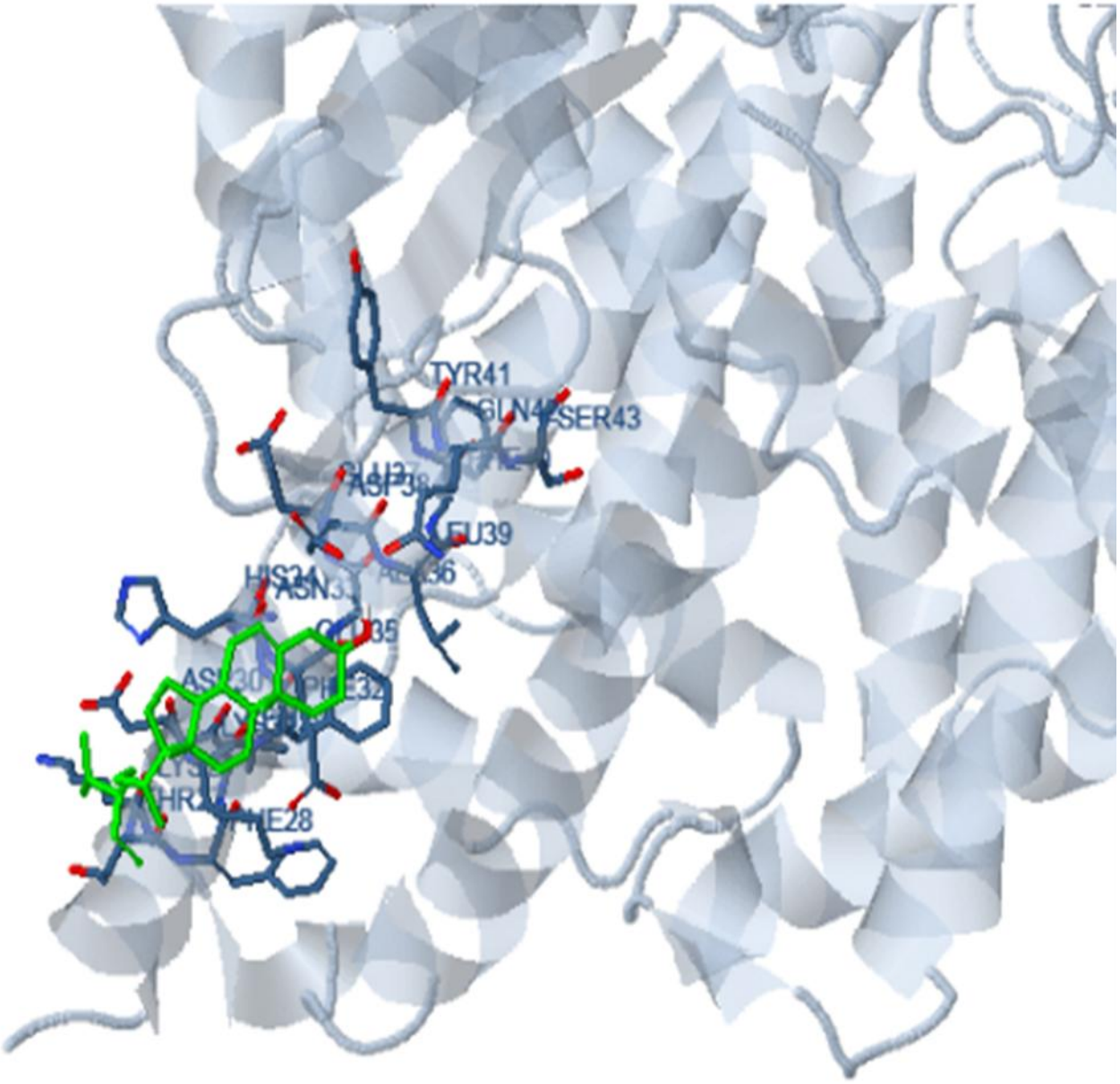
2D Interaction Plot



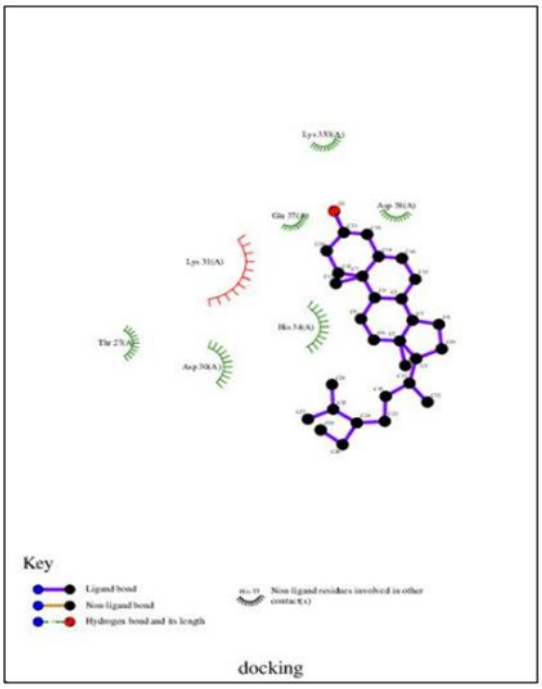
Hydrogen bond plotting with core amino acid Analysis



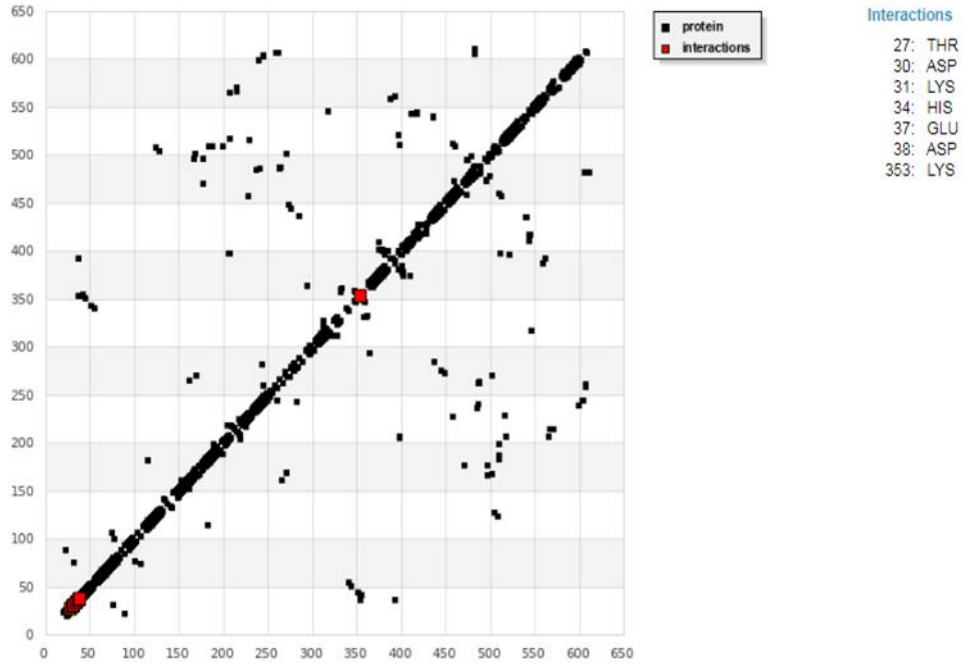
Docking Pose
β-sitosterol with Angiotensin-converting enzyme 2 (ACE2) receptor- PDB 2AJF



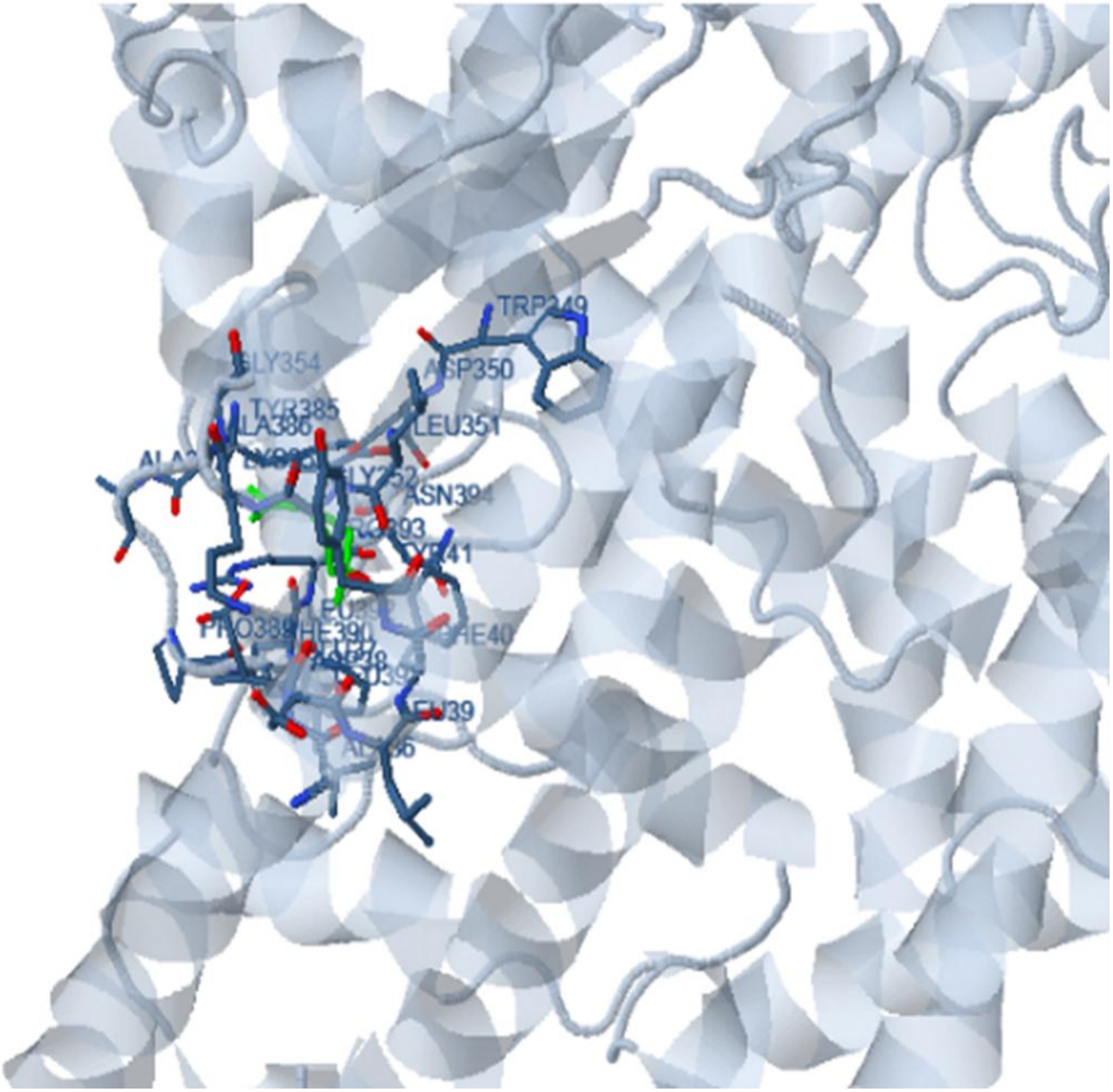
2D Interaction Plot



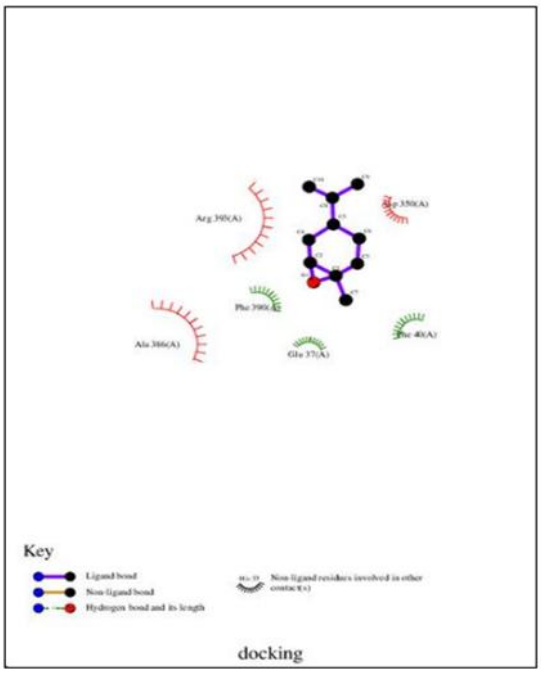
Hydrogen bond plotting with core amino acid Analysis



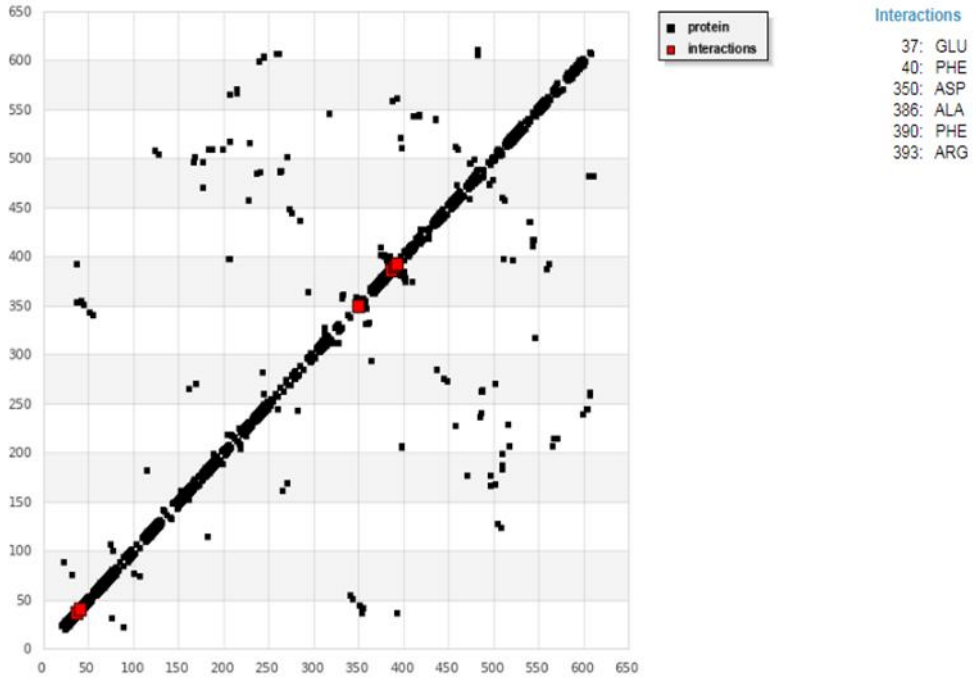
Docking Pose
Limonene with Angiotensin-converting enzyme 2 (ACE2) receptor- PDB 2AJF



2D Interaction Plot



Hydrogen bond plotting with core amino acid Analysis



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