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REVEALING ANTI-VIRAL POTENTIAL OF VELLAI ERUKKAN SAMULA PARPAM TARGETING SARS-COV2 – POLYMERASE (RdRp) IN COMBATING COVID-19: MOLECULAR INVESTIGATION ON SIDDHA TRADITIONAL MEDICINE

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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 RdRp. 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 (618 ASP, 760 ASP, 761 ASP) of the targets by forming hydrogen bond will hinder the function of the targets RNA dependent RNA polymerase (PDB)-6NUR possess versatile action in mediating nonstructural protein (nsp 12) essential for viral replication. Total of 8 bioactive lead compounds from reported data of the herb, the lead compound's such as Rutin, Quercetin 3-O-galactoside, Calotropagenin, Calotropin, Uscharidin, Coroglaucigenin, β-sitosterol and R-limonene possess 100% binding efficacy by interacting with all three core target amino acid present on the target receptor RdRp. Conclusion: Based on the results of the computational analysis it was concluded that the compound's such as Rutin, Quercetin 3-O-galactoside, Calotropagenin, Calotropin, Uscharidin, Coroglaucigenin, β-sitosterol and R-limonene present in the herbal ingredients of the formulation Vellai Erukkan Samula Parpam revels significant binding against the target protein thereby it was concluded that these compounds exerts promising inhibiting against RdRp enzyme and thereby halt the viral replication. 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.

KEY WORDS: ***Vellai Erukkan Samula Parpam (Calotropis Procera), Anti-Viral Herbs, Siddha Medicine, SARS-CoV-2 COVID-19, RNA dependent RNA polymerase (RdRp), In-Silico Molecular Docking Analysis.***

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1. 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).

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).

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 RNA dependent RNA polymerase (RdRp).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 (618 ASP, 760 ASP, 761 ASP) of the targets by forming hydrogen bond will hinder the function of the targets RNA dependent RNA polymerase (PDB)-

6NUR possess versatile action in mediating nonstructural protein (nsp 12) essential for viral replication. Thereby phytocomponents which inhibit the target RdRp may act as a potential therapeutic agent for management of COVID-19 and related symptoms.

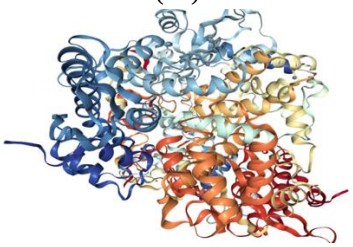
6NUR - RNA dependent RNA polymerase (RdRp)

2.Materials and Methods

Docking calculations were carried out for retrieved phytocomponents against target protein RdRp. 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 RNA dependent RNA polymerase (PDB)-6NUR

Crystalline structure of the target protein RNA dependent RNA polymerase (PDB)-6NUR 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.

வெள்ளெருக்கு (2)	CALOTROPIS PROCERA	WHOLE PLANT

3.Results

3.1. Observation and Inference

Total of 8 bioactive lead compounds from reported data of the herb, the lead compound's such as Rutin, Quercetin 3-O-galactoside, Calotropagenin, Calotropin, Uscharidin, Coroglaucigenin, β-sitosterol and R-limonene possess 100% binding efficacy by interacting with all three core target amino acid present on the target receptor RdRp.

Binding of phytocomponents with the core amino acids (618 ASP, 760 ASP, 761 ASP) of the targets by forming hydrogen bond will hinder the function of the targets RNA dependent RNA polymerase (PDB)-6NUR possess versatile action in mediating nonstructural protein (nsp 12) essential for viral replication. Thereby phytocomponents which inhibit the target RdRp may act as a potential therapeutic agent for management of COVID-19 and related symptoms.

4.Discussion and Conclusion

Based on the results of the computational analysis it was concluded that the compound's such as Rutin, Quercetin 3-O-galactoside, Calotropagenin, Calotropin, Uscharidin, Coroglaucigenin, β-sitosterol and R-limonene present in the herbal ingredients of the formulation Vellai Erukkan Samula Parpam revels significant binding against the target protein thereby it was concluded that these compounds exerts promising inhibiting against RdRp enzyme and thereby halt the viral replication. 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.

This work is carried out based on the symptoms given by WHO for COVID 19 based on the Siddha classical text. To the motive of giving helping hand to our

beloved nation under this catastrophic situation. We request the concern personality to take further research on the drugs selected for the symptoms and save lives. The plant Calotropis procera have been researched more on toxicity, pharmacology evidence(Anti-Viral activity, Anti-Angiogenic activity, Bronchodilator Activity, Immunomodulatory activity, Anti-pyritic activity, Anti-Microbial activity, Anti-Cancer ,Anti-Histaminic activity, Anti-Convulsant activity)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 various respiratory symptoms like dyspnoea, shortness of breathing, Chest discomfort, Wheezing, breathlessness, cold , Cough with tenacious sputum and other respiratory diseases.

I hereby conclude that the many pharmacological and toxicity studies have been already published in various peer review journals about Calotropis procera and also the literary evidences support the usage of drugs in the disease, 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. like acute, chronic respiratory illness , viral diseases, also for all other respiratory diseases and so the permission may be given for conducting the clinical trial to validate the therapeutic effect of the drug and it will pave the way for promoting the wellness of individuals.

Utilization of outcomes of project

The purpose of the proposal of this Siddha trial medicine 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 effective in reduction of clinical symptoms and in the management and for enhancing the immune system for against any viral infection.

Acknowledgement

I wish to acknowledge my sincere thanks to The Tamil Nadu Dr. M.G.R. Medical University, Chennai, Tamil Nadu, India and The Noble research solutions, Chennai, Tamil Nadu, India for their support.

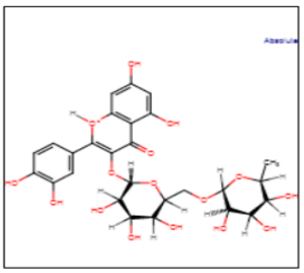
6. References

1. Yan-Rong Guo et.al, The origin, Transmission and clinical therapies on coronavirus disease 2019 (COVID-19) outbreak – An update on the status, Military medical research, 2020.
2. 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).
3. Clinical management of severe acute respiratory infection (SARI) when COVID-19 disease is suspected, interim guidance, WHO, March 2020.
4. Marco Cascella et al, Features, Evaluation and Treatment Coronavirus (COVID-19), March 2020.
5. Kuppusamy muthaliyaar.K.N (2016), Siddha Maruthuvam (pothu), 8th edition, Pg.no : 33-35, 65.
6. Thiyagarajan (1976), Yugi Munivar Vaithiya Chinthamani, 2nd edition, Pg no: 100, 107, 478.
7. Bikadi, Z., Hazai, E.Application of the PM6 semi-empirical method to modeling proteins enhances docking accuracy of AutoDock. J. Cheminf. 1, 15 (2009)
8. T. A. Halgren. Merck molecular force field. I. Basis, form, scope, parametrization, and performance of MMFF94. Journal of Computational Chemistry 17 (5-6), 490-519 (1998)
9. G. M. Morris, D. S. Goodsell, et al. Automated docking using a Lamarckian genetic algorithm and an empirical binding free energy function. Journal of Computational Chemistry 19 (14), 1639-1662(1998)
10. F. J. Solis and R. J. B. Wets. Minimization by Random Search Techniques.
11. AMY.Moustafa et al. Extraction and phytochemical investigation of Calotropis procera: Effect of plant extracts on the activity of diverse muscles. Journal of Pharmaceutical Biology. 2010;48:1080-1190.
12. Sony J.Chundattu. Phytochemical investigation of Calotropis procera. Arabian Journal of Chemistry. Volume 9, Supplement 1, September 2016, Pages S230-S234.
13. Reena Rani Et al. Phytochemical Analysis, Antibacterial and Antioxidant Activity of Calotropis procera and Calotropis gigantean. The Natural Products Journal.2019;9(1):1-16.

2D and 3D Structure of Selected Ligands

Rutin

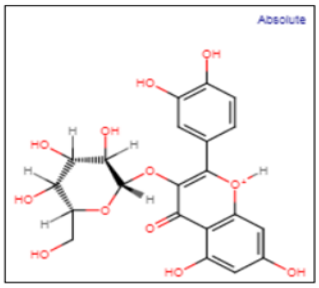
Ligand in 2D



Ligand in 3D

Quercetin 3-O-galactoside

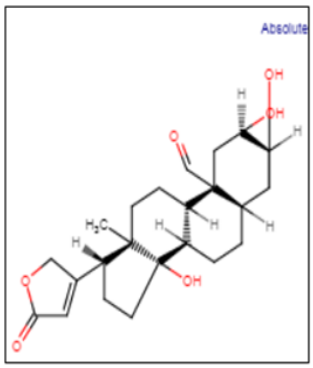
Ligand in 2D



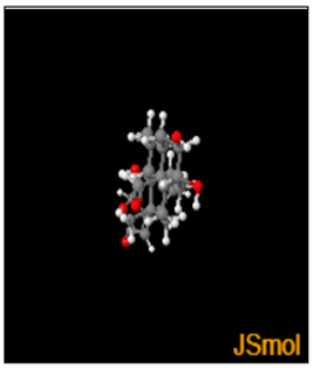
Ligand in 3D

Calotropagenin

Ligand in 2D

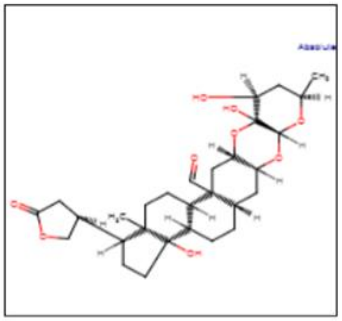


Ligand in 3D



Calotropin

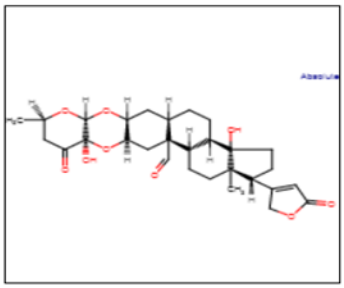
Ligand in 2D



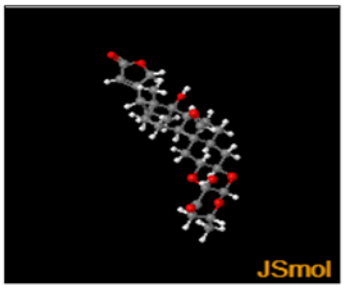
Ligand in 3D

Uscharidin

Ligand in 2D

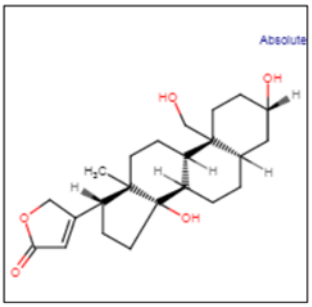


Ligand in 3D



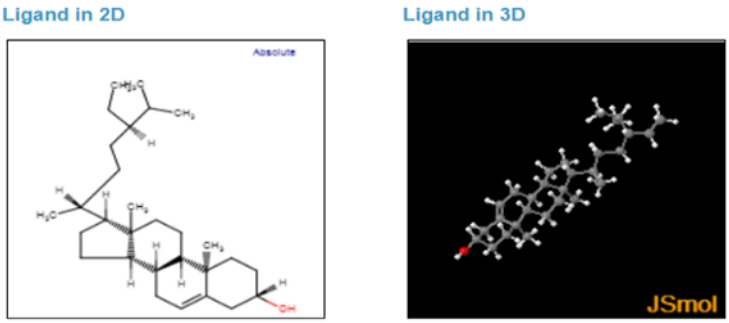
Coroglaucigenin

Ligand in 2D



Ligand in 3D

β-sitosterol



R-limonene

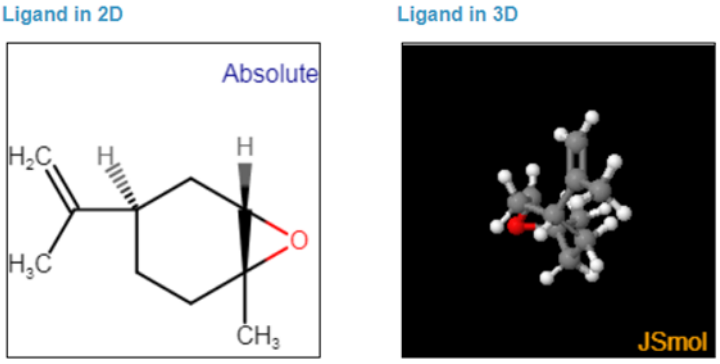


Table 1: Ligand Properties of the Compounds Selected for Docking Analysis

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	$C_{10}H_{16}$	0	0	1

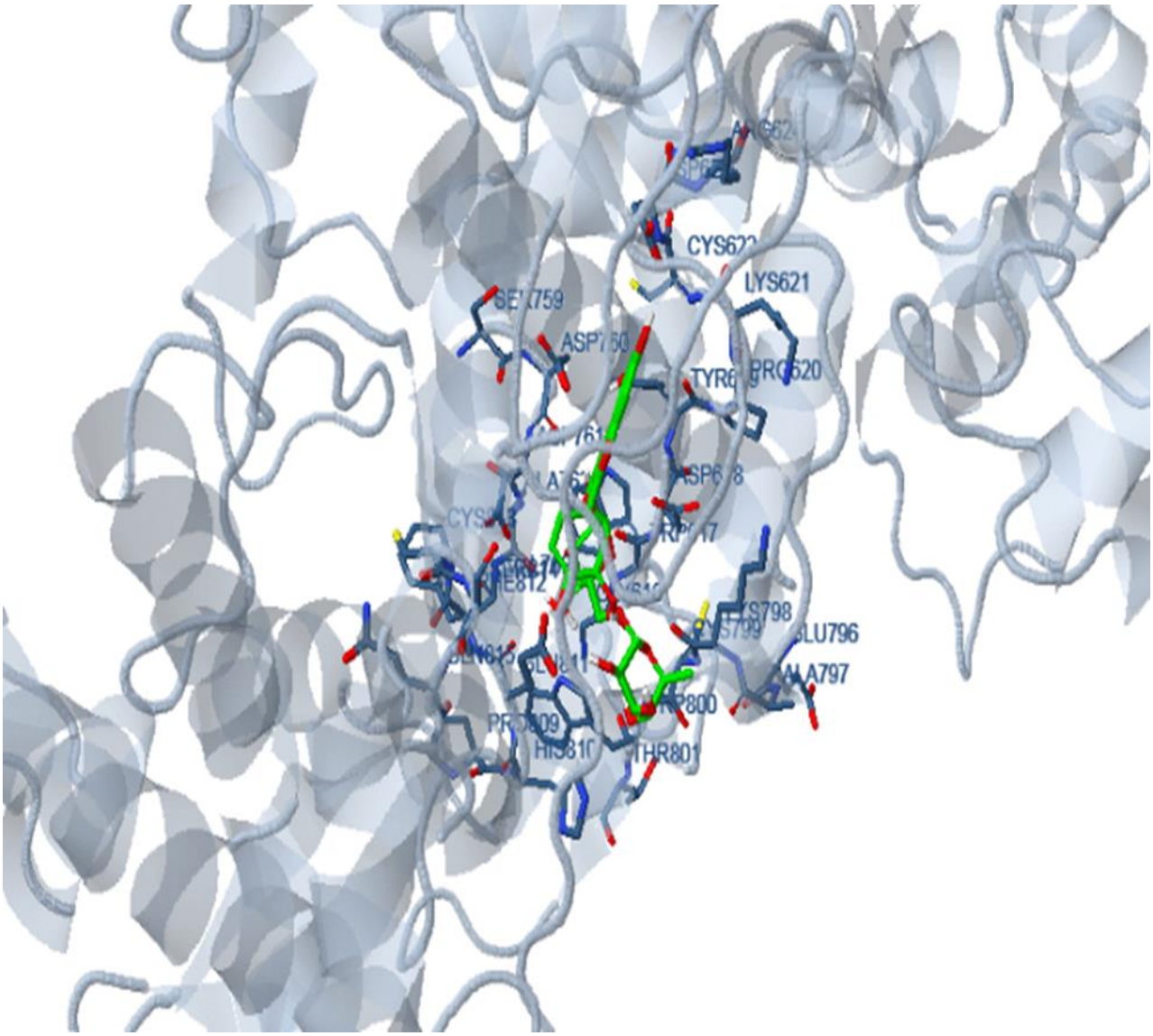
Table 2: Summary of the molecular docking studies of compounds against RNA dependent RNA polymerase (PDB)-6NUR

Compounds	Binding Free energy Kcal/mol	Inhibition constant Ki µM (*mM)(**nM)	Electrostatic energy Kcal/mol	Intermolecular energy Kcal/mol	Total Interaction Surface
Rutin	-4.55	459.62	-0.98	-7.59	909.88
Quercetin 3-O-galactoside	-9.53	103.71**	-0.87	-7.46	783.09
Calotropagenin	-6.36	21.88	-0.41	-6.98	646.85
Calotropin	-7.74	2.13	-0.33	-8.06	822.88
Uscharidin	-6.30	24.49*	-0.23	-7.01	860.61
Coroglaucigenin	-7.32	4.34*	-0.73	-7.52	715.84
β-sitosterol	-7.95	1.49*	-0.09	-9.15	757.53
Limonene	-4.70	355.94	-0.04	-5.00	455.86

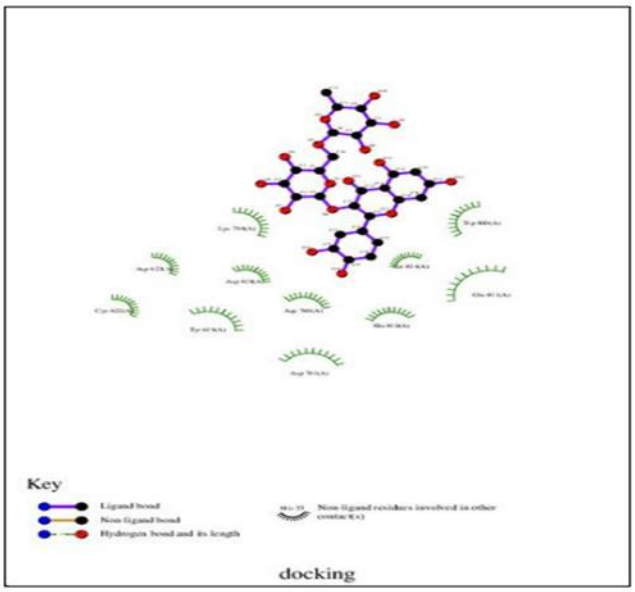
Table 3: Amino acid Residue Interaction of Lead against RNA dependent RNA polymerase (PDB)-6NUR

Molecule	Interactions	Amino Acid Residue- Binding										
Rutin	3	618 ASP	619 TYR	622 CYS	623 ASP	760 ASP	761 ASP	798 LYS	800 TRP	810 HIS	811 GLU	814 SER
Quercetin 3-O-galactoside	3	618 ASP	619 TYR	622 CYS	758 LEU	760 ASP	761 ASP					
Calotropagenin	3	618 ASP	760 ASP	761 ASP	800 TRP	811 GLU	814 SER					
Calotropin	3	618 ASP	758 LEU	760 ASP	761 ASP	800 TRP	811 GLU	813 CYS	814 SER	836 SRG		
Uscharidin	3	618 ASP	623 ASP	687 THR	691 ASN	760 ASP	761 ASP	800 TRP	811 GLU	814 SER		
Coroglaucigenin	3	618 ASP	619 TYR	622 CYS	623 ASP	760 ASP	761 ASP					
β-sitosterol	3	618 ASP	758 LEU	759 SER	760 ASP	761 ASP	800 TRP	811 GLU	814 SER			
R-limonene	3	618 ASP	761 ASP	800 TRP								

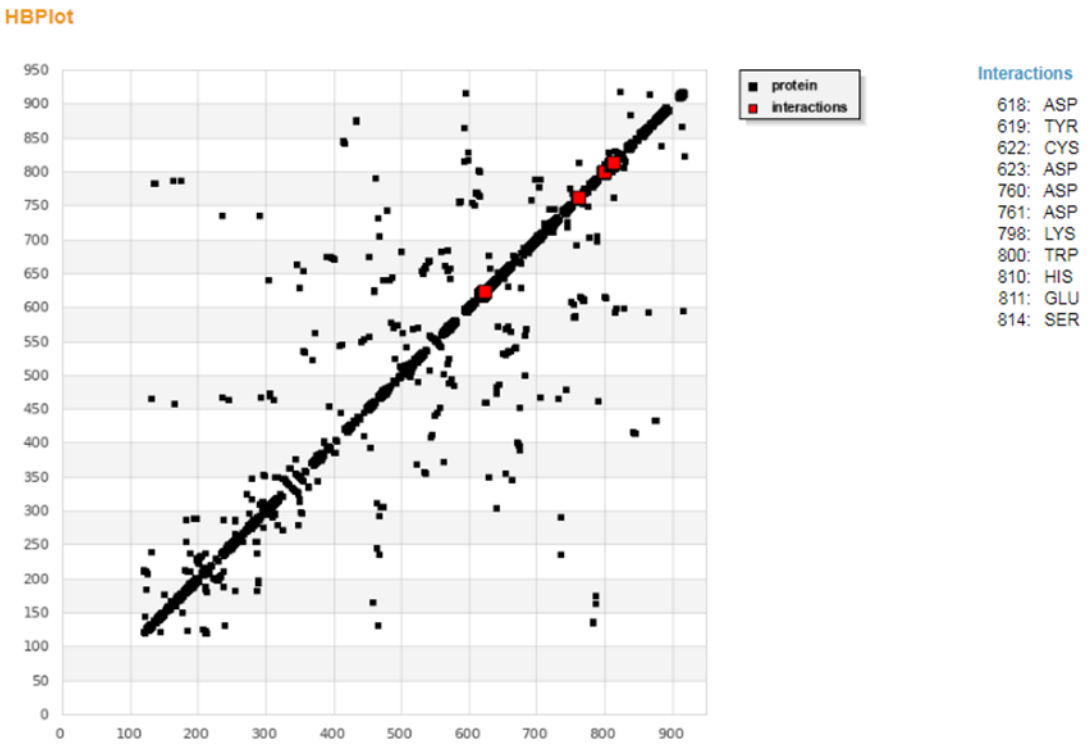
Rutin with RNA dependent RNA polymerase- PDB 6NUR



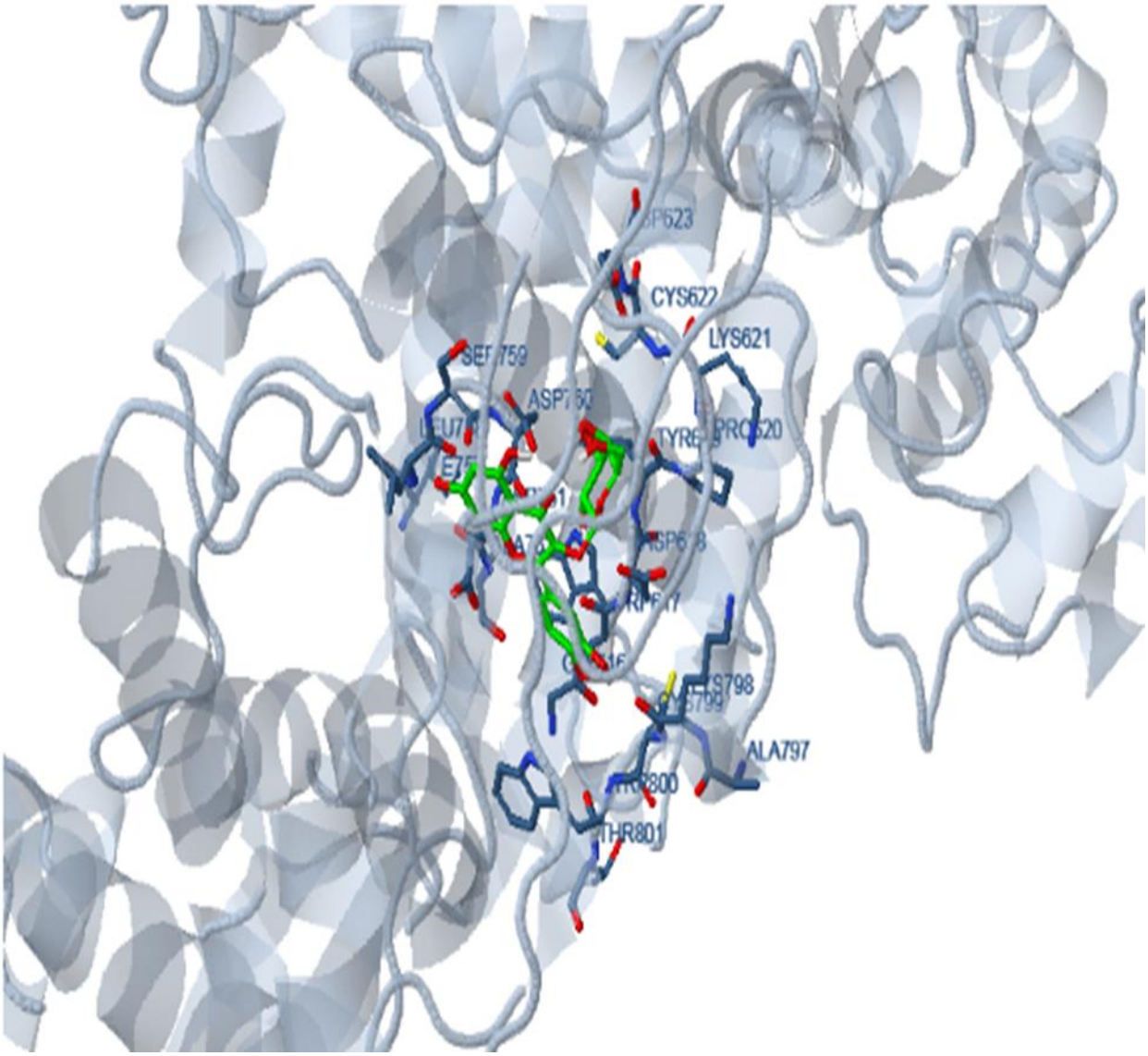
2D Interaction Plot



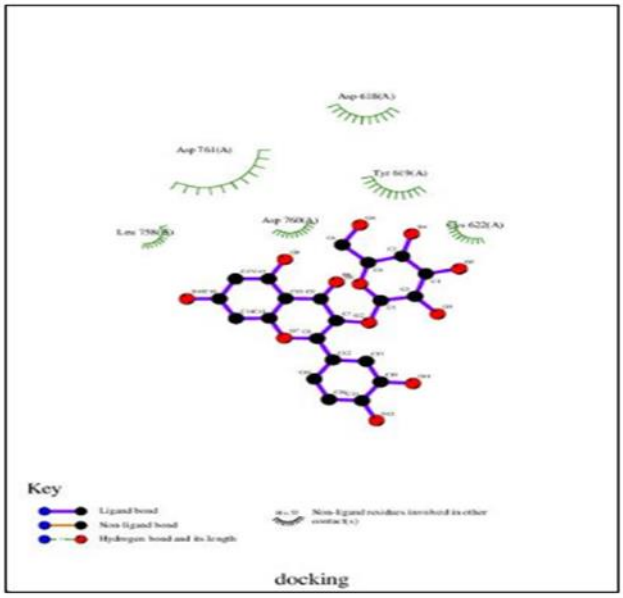
Hydrogen bond plotting with core amino acid Analysis



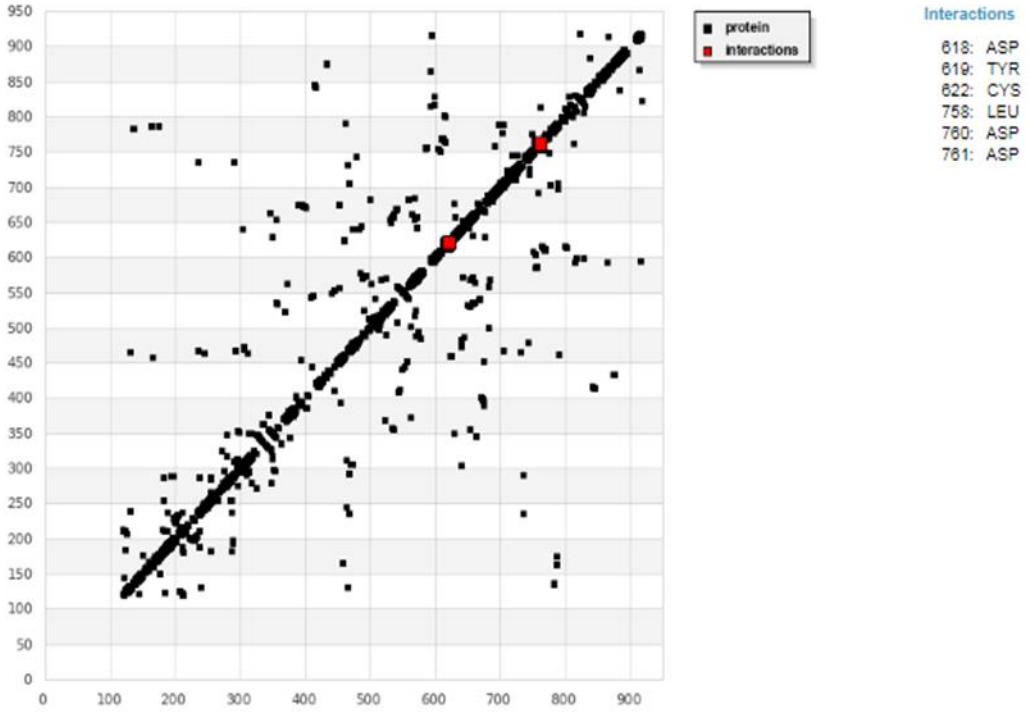
Quercetin 3-O-galactoside with RNA dependent RNA polymerase- PDB 6NUR



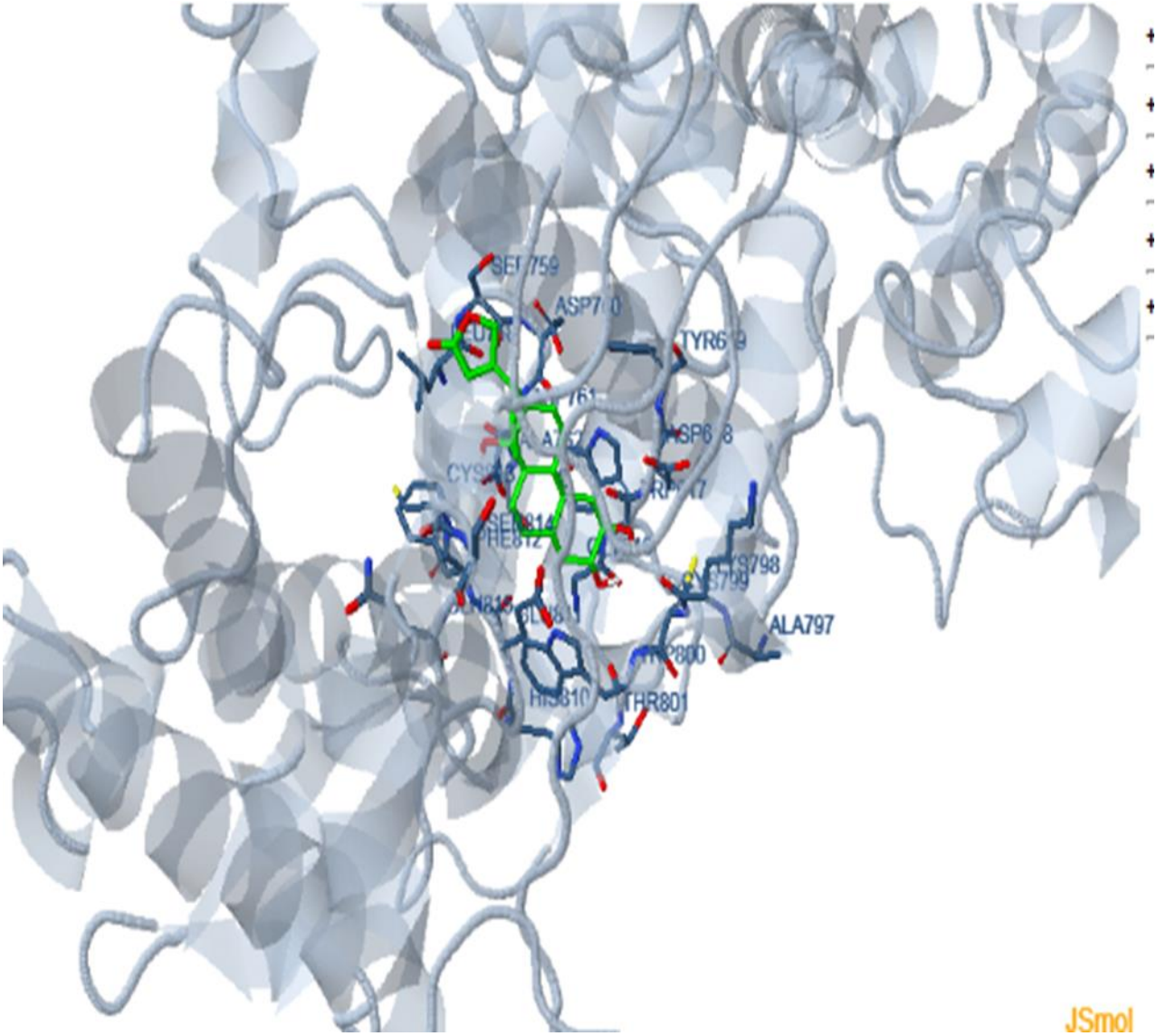
2D Interaction Plot



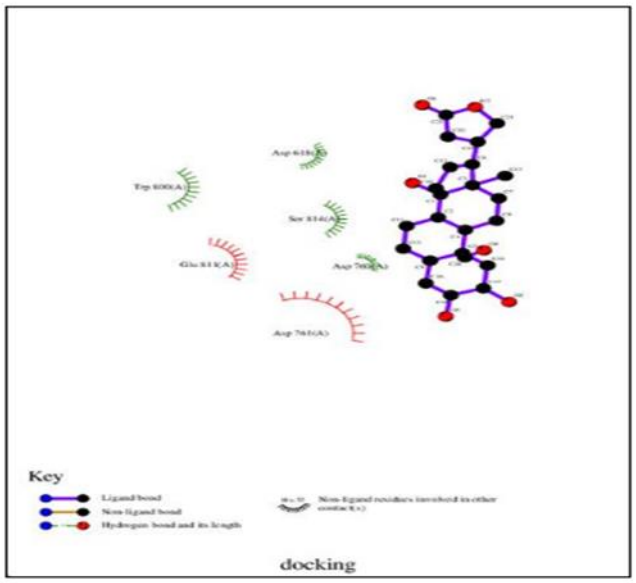
Hydrogen bond plotting with core amino acid Analysis



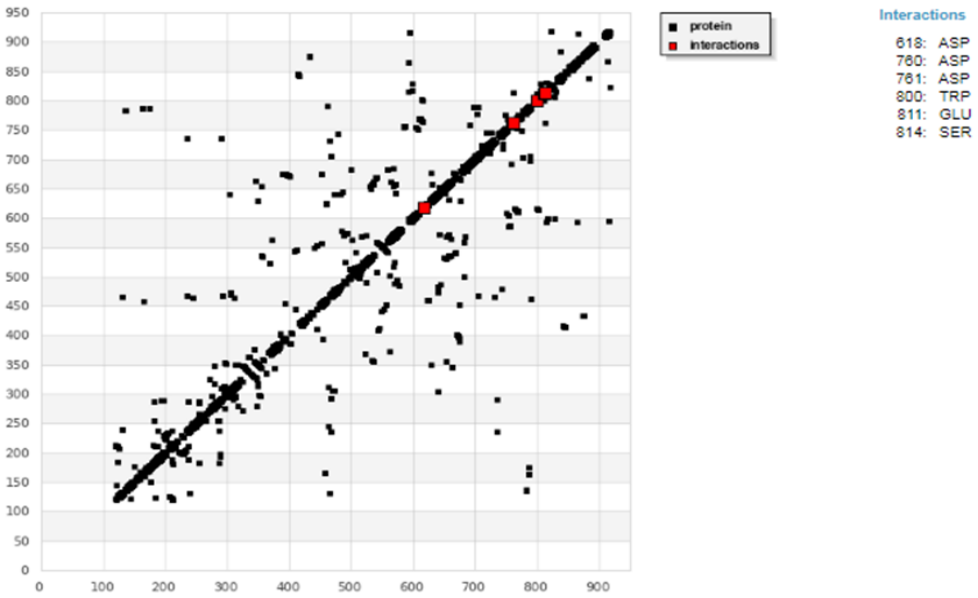
Calotropagenin with RNA dependent RNA polymerase- PDB 6NUR



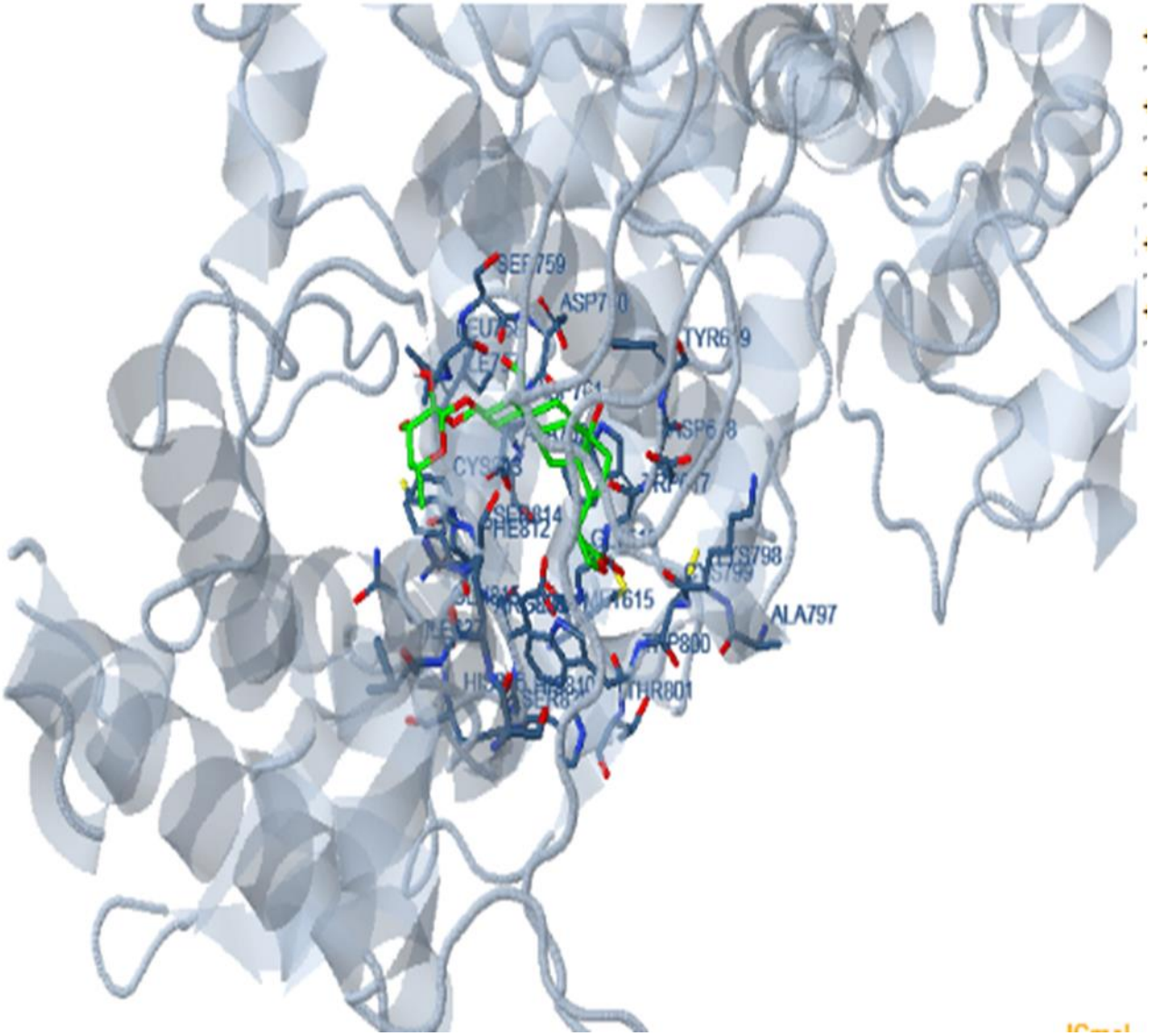
2D Interaction Plot



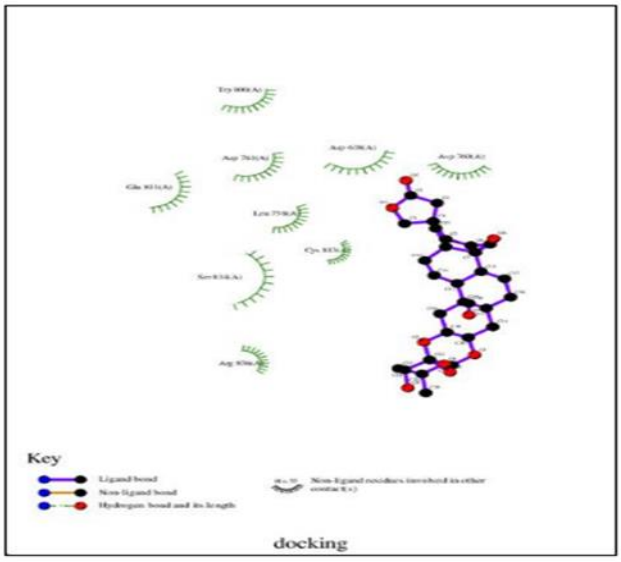
Hydrogen bond plotting with core amino acid Analysis



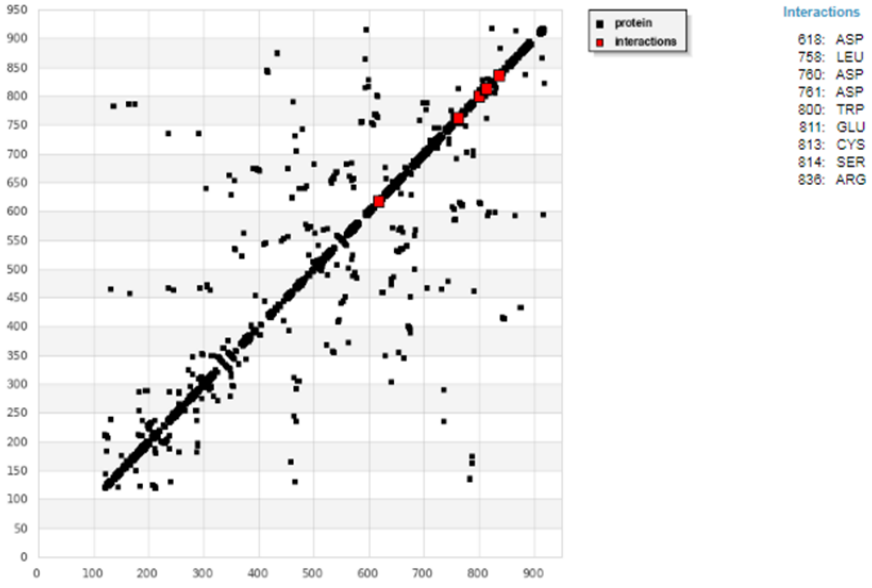
Calotropin with RNA dependent RNA polymerase- PDB 6NUR



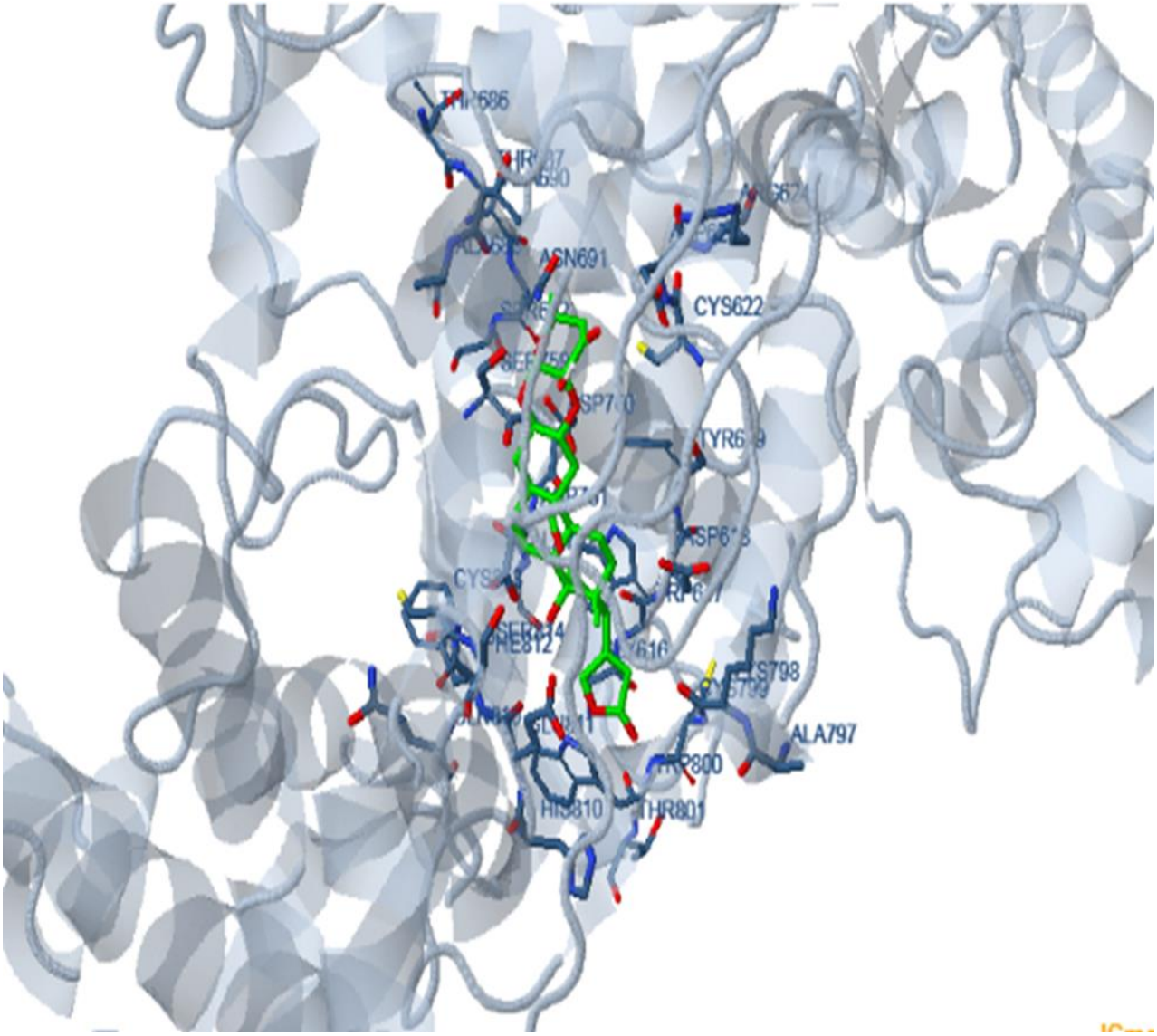
2D Interaction Plot



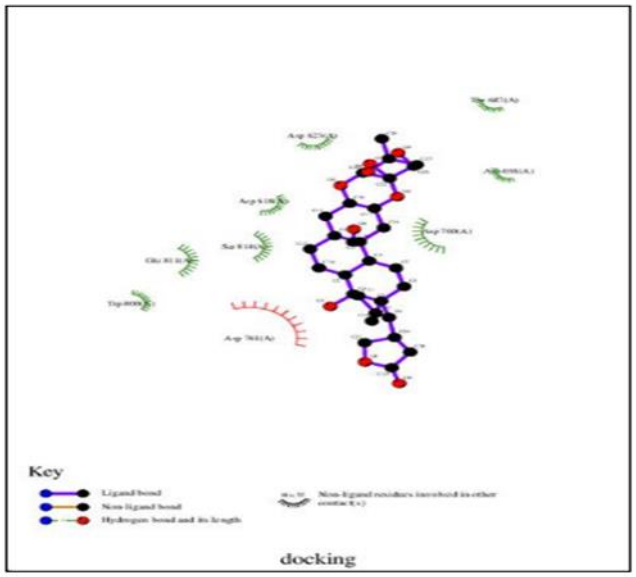
Hydrogen bond plotting with core amino acid Analysis



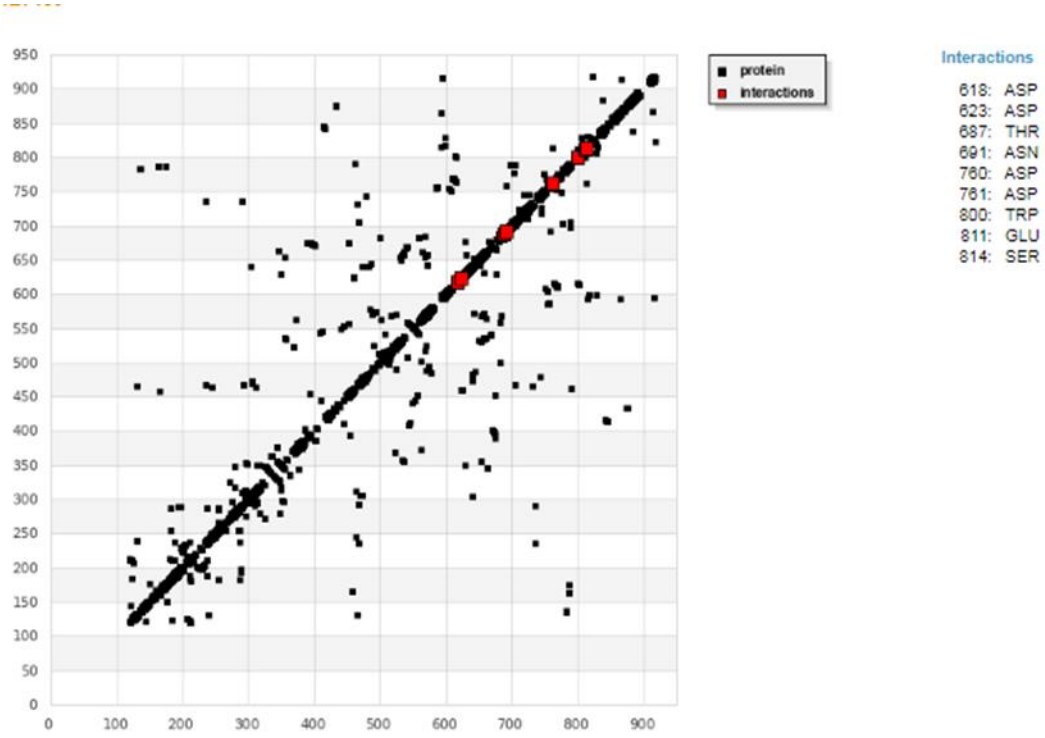
Uscharidin with RNA dependent RNA polymerase- PDB 6NUR



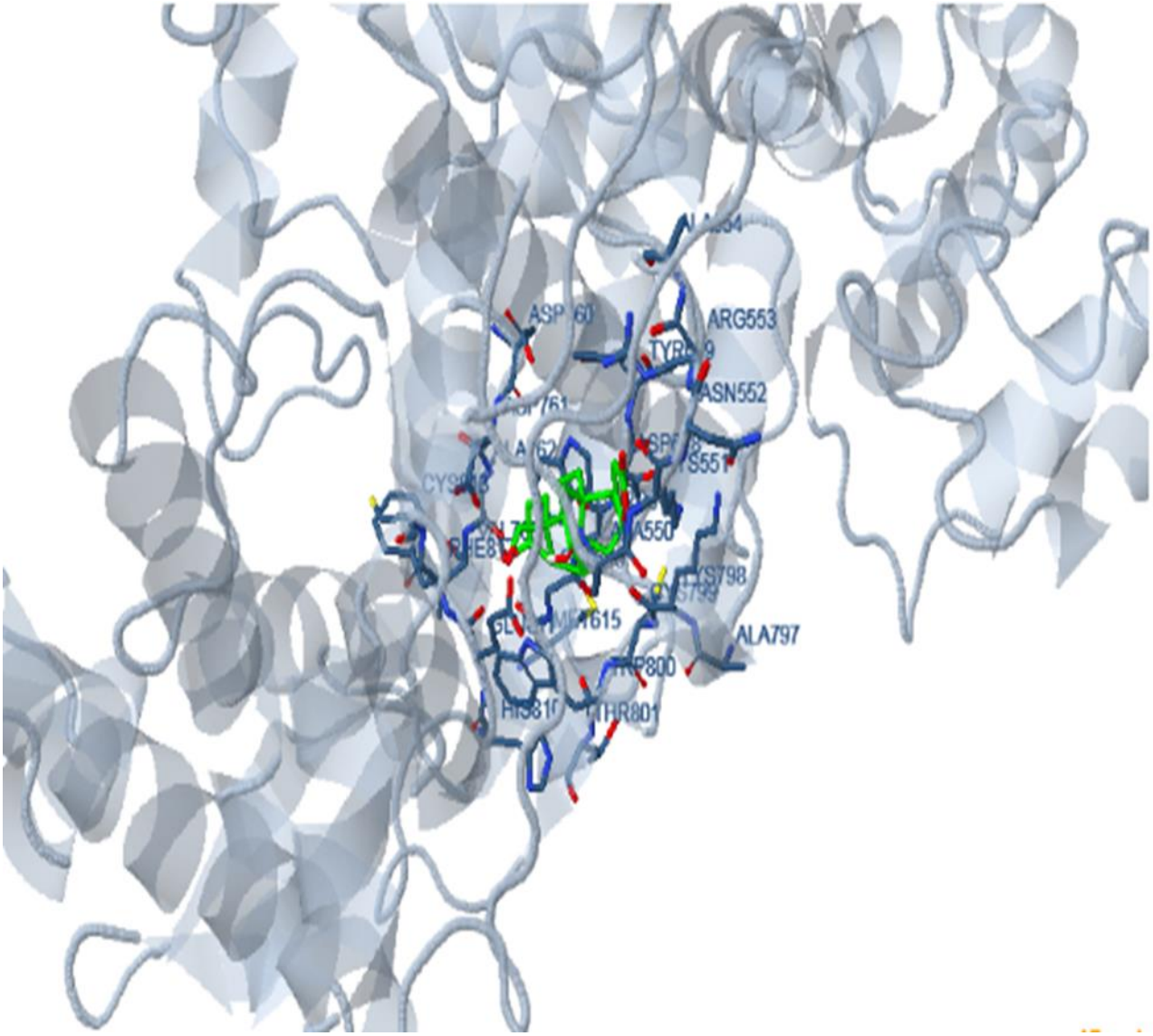
2D Interaction Plot



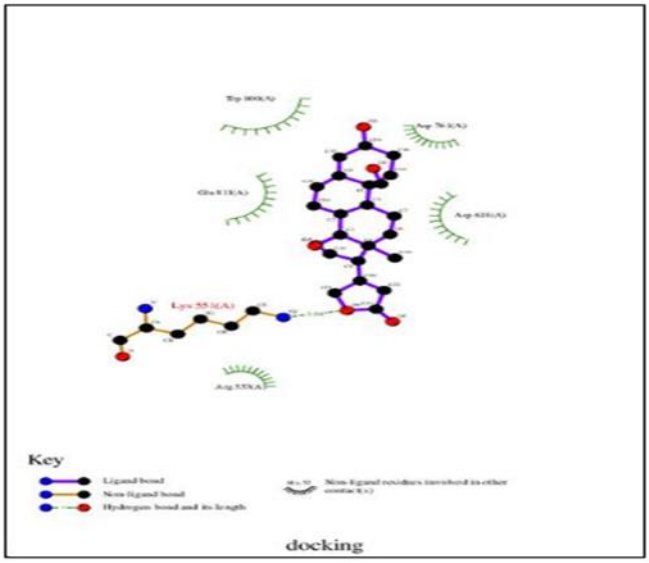
Hydrogen bond plotting with core amino acid Analysis



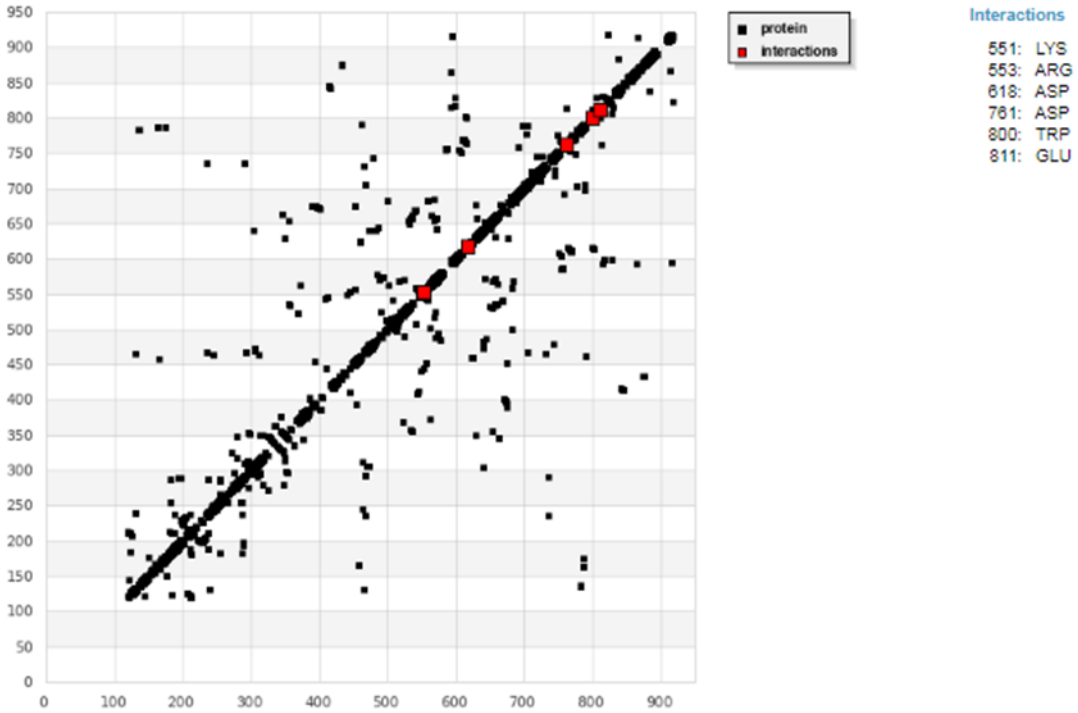
Coroglaucigenin with RNA dependent RNA polymerase- PDB 6NUR



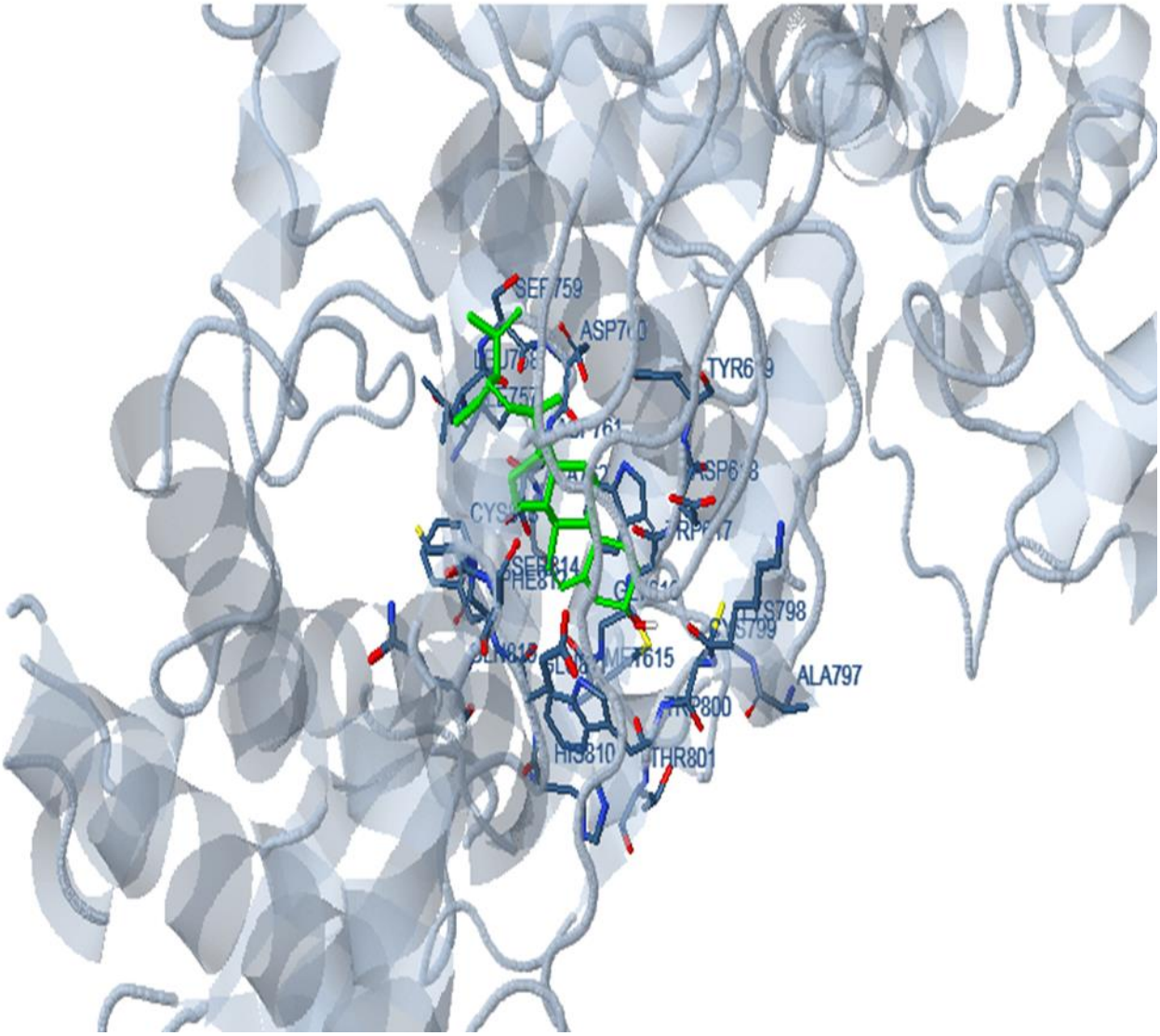
2D Interaction Plot



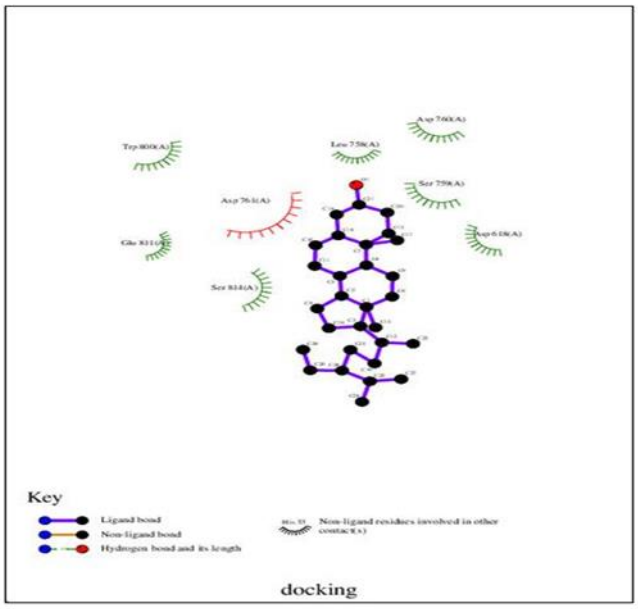
Hydrogen bond plotting with core amino acid Analysis



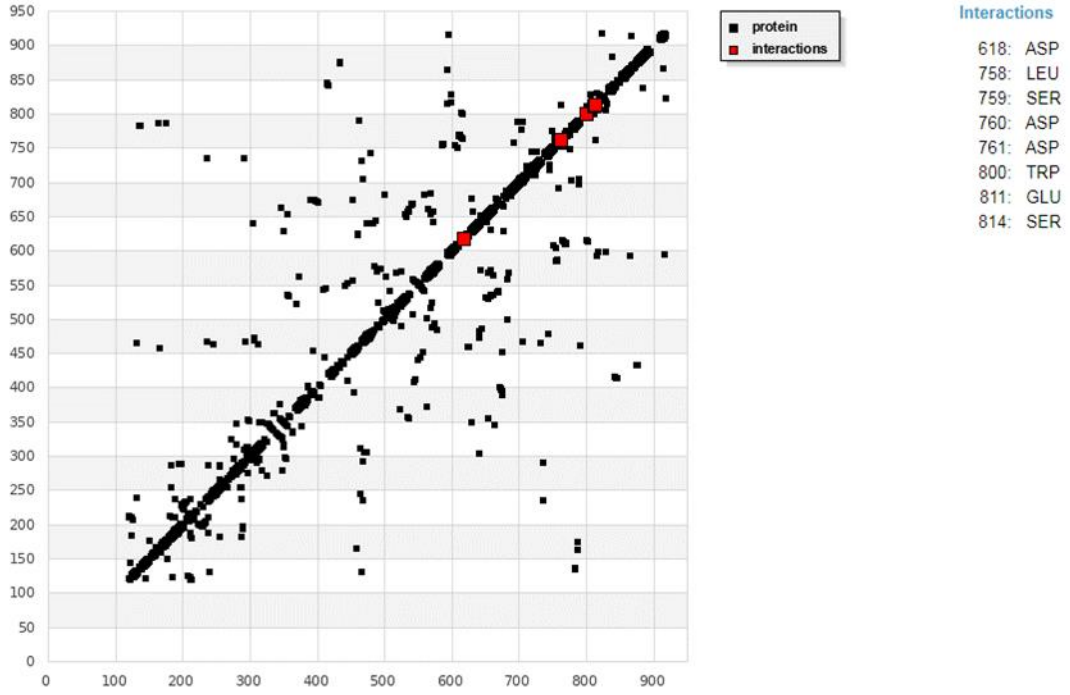
β-sitosterol with RNA dependent RNA polymerase- PDB 6NUR



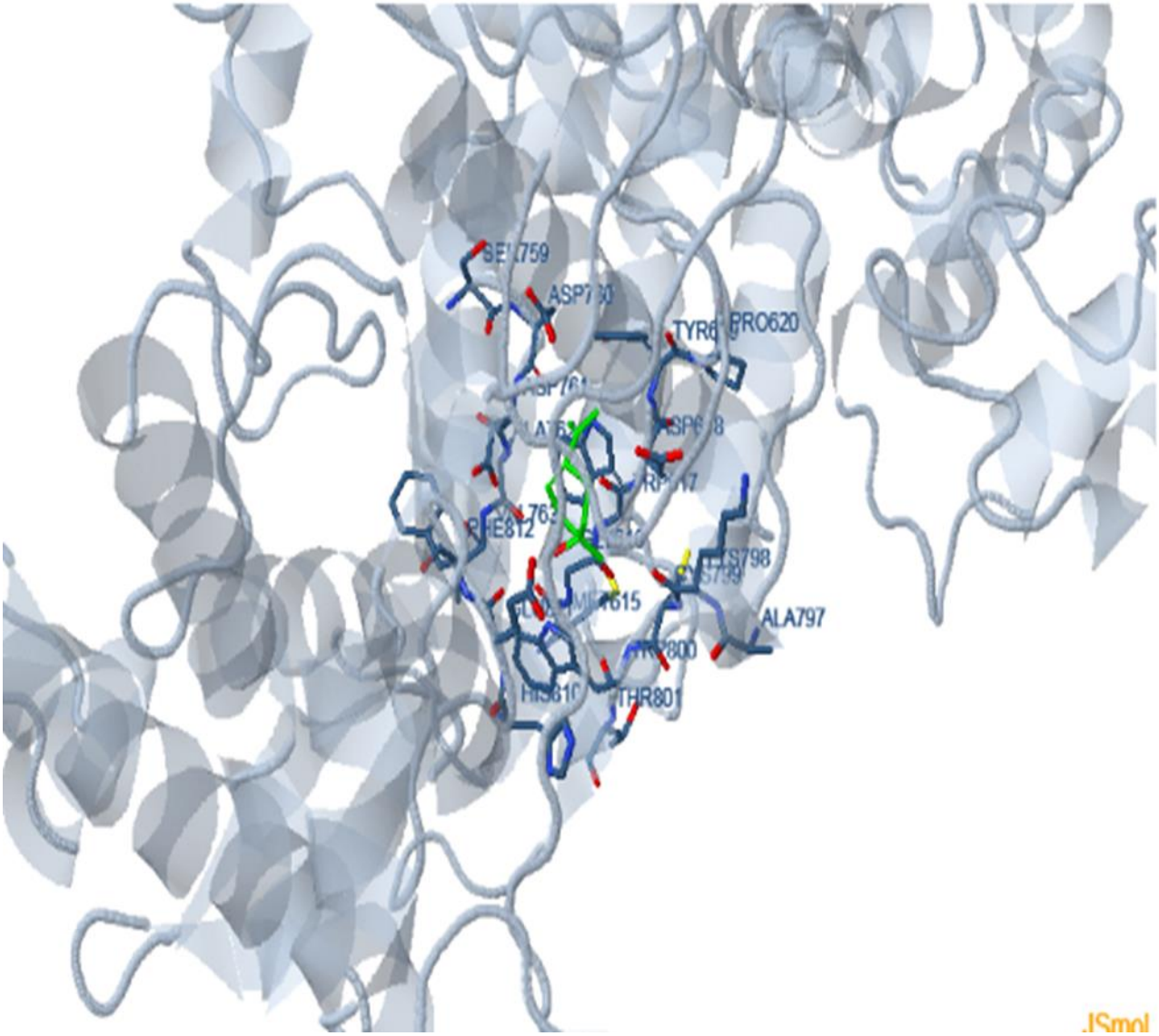
2D Interaction Plot



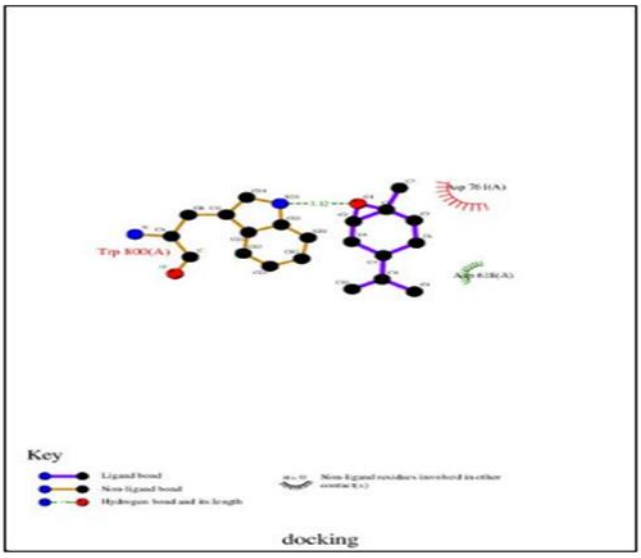
Hydrogen bond plotting with core amino acid Analysis



Limonene with RNA dependent RNA polymerase- PDB 6NUR



2D Interaction Plot



Hydrogen bond plotting with core amino acid Analysis