INTERNATIONAL JOURNAL OF CURRENT RESEARCH IN BIOLOGY AND MEDICINE
ISSN: 2455-944X
www.darshanpublishers.com
Volume 5, Issue 11 - 2020

Original Research Article

DOI: http://dx.doi.org/10.22192/ijcrbm.2020.05.11.001

In-Silico Evaluation of Anti-Viral Potential of Siddha herbal Formulation *Moringa oleifera* (Murungai) against 3-CLpro Enzyme target in the treatment of SARS Co-V-2 (COVID-19)

M. K. Sathesh Kumar*[1], P. Sharmila[2], P. Jayapriya[3], T. Lakshmikantham[4], R. Meenakumari[5]

[1&2] P.G Scholar, Department of Maruthuvam, National Institute of Siddha, Tambaram Sanatorium, Chennai-6000047, Tamil Nadu, India
[3] P.G Scholar, Department of Kuzhandai Maruthuvam, National Institute of Siddha, Tambaram Sanatorium, Chennai-6000047,Tamil Nadu, India
[4]Head of the Department, Department of Maruthuvam, National Institute of Siddha Tambaram Sanatorium, Chennai-6000 047,Tamil Nadu, India.
[5]Director, Professor and Head of the Department, Department of Gunapadam, National Institute of Siddha, Chennai- 6000047, Tamil Nadu, India.

Corresponding Address: **M. K. Sathesh Kumar,** P.G. Scholar, Department of Maruthuvam, National Institute of Siddha, Tambaram Sanatorium, Chennai 6000 047, Tamil Nadu, India.

Abstract

Infections becomes a regular part of the human life, as it is hypothesizes that ever since from the birth humans are constantly exposed to the wide spectrum of microbial infestation. One such recent infectious paradigm caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is novel corona virus disease (COVID-19). Due to limitation prevails in availing the allopathic drugs there is a constant drive for the researchers to strive for alternate therapeutic strategy for controlling the pandemic spread of COVID-19. Herbs like *Moringa oleifera* traditionally used for treating various diseases and disorders since centuries. The main aim of the present investigation is to explore the anti-viral potential of the phytocomponents such as Ascorbic acid, Moringine, Beta-Sitosterol, Nicotinic acid, Zeatin, Alpha tocopherol, Quercetin, Chlorogenic acid and Kaemferol present in the herb *Moringa oleifera* against the enzyme target 3-chymotrypsin-like protease ($3CL^{pro}$)by using AutoDock prediction. Results of molecular docking analysis strongly suggested that out of nine phyto components leads such as Beta-Sitosterol, Zeatin, Alpha tocopherol, Quercetin and Chlorogenic acid present in the herb *Moringa oleifera* revels significant binding against the target protein $3CL^{pro}$ thereby it was concluded that these compounds may exerts promising inhibiting against $3CL^{pro}$ enzyme and hereby halt the formation of 16 non-structural proteins (nsp1-nsp16) that are highly essential for viral replication and there by prevents the viral survival in the host environment.

Keywords: COVID-19, SARS-CoV-2, $3CL^{pro}$, *Moringa oleifera*, Phytocomponents, Docking

1. Introduction

Coronaviruses infect the upper gastrointestinal and respiratory tract of the mammals (including humans) and the birds. These viruses cause many diseases in animals and human beings but we are limited in this article with SARS-CoV-2, leading to COVID-19 disease. The whole clinical picture of COVID-19 is not completely known. The occurrence of the illness ranged from mild to severe. SARS-CoV-2 propagate through RNA replication using RNA-dependent RNA polymerases enzyme. This virus can mutate slowly, posing a challenge for its treatment and control. The symptoms of COVID-19 may arise within 2 to 14 days after the infection [1].

The $3CL^{pro}$ cleaves the polyprotein at 11 distinct sites to generate various non-structural proteins that are important for viral replication [2]. $3CL^{pro}$ plays a critical role in the replication of virus particles and unlike structural/accessory protein-encoding genes, it is located at the 3′ end which exhibits excessive variability. Therefore, it is a potential target for anti-coronaviruses inhibitors screening [3]. Structure-based activity analyses and high-throughput studies have identified potential inhibitors for SARS-CoV and MERS-CoV $3CL^{pro}$ [4].

In the past few decades, natural products have been an important source of potential drug hits and leads [5]. However, development efforts in NP drug discovery have demonstrated a certain downturn in recent years [6]. Despite this decline, the vast chemical space of natural products continues to provide abundant structural diversity for discovering novel lead compounds with low molecular weight. Less than 10% of the world's biodiversity has been explored to find potential biologically active compounds [7]

Herbs like *Moringa oleifera* traditionally used for the management of infective and degenerative disorders. This unique herb posses several structurally versatile components like Moringine, Beta-Sitosterol, Nicotinic acid, Zeatin, Alpha tocopherol, Quercetin, Chlorogenicacid and Kaemferol. Each of these leads is already well explored for its efficacy against several diseases and disorders.

Molecular docking is frequently used in the process of computer aided drug design (CADD). It can be applied in different stages of the drug design process in order to predict the binding mode of already known ligands [8]; identify novel and potent ligands [9] and as a binding affinity predictive tool [10]. Virtual screening is nowadays a method of choice to search large databases of compounds and to select compounds for in vitro testing. Virtual screening approaches can be divided into ligand-based and structure-based. When the 3D structure of a target is known from experimental or computational studies, high-throughput docking is a method of choice. The alternative method is pharmacophore-based virtual screening.

2. Materials and Methods

2.1. Protein-ligand docking

Computational molecular investigation was performed using Auto Dock version 4 which predicts interaction binding affinity between selected therapeutic lead with that of the protein target COVID-19 main protease (3-chymotrypsin-like protease (3CL pro)) -PDB- 6LU7.

2.2. Protein preparation

Three dimensional (3D) structure of COVID-19 main protease (3-chymotrypsin-like protease 3CL pro with protein data bank (PDB)-6LU7 (Figure 1) retrieved from Research Collaboratory for Structural Bioinformatics (RCSB) [11].

2.3. Ligand model preparation

Structures of the phyto components such as Moringine, Beta-Sitosterol, Nicotinic acid, Zeatin, Alpha tocopherol, Quercetin, Chlorogenic acid and Kaemferol subjected to docking investigation were outlined using ChemDraw sketch software and converted from two dimension (2D) to3D structures. Figure 2 summarizing 2D and 3D structure of approved ligand subjected to molecular docking Investigation against COVID-19 main protease (3-chymotrypsin-like protease 3CL pro with protein data bank (PDB)-6LU7.

2.4. Docking simulations

Molecular docking analysis were performed using licensed version of Auto Dock 4, which predicts interactions between FDA approved drug molecules with that of the selected protein target (Novel coronavirus 3-chymotrypsin-like protease (3CL pro). 3D structure of main protease that is 3- chymotrypsin-like protease (3CL pro) with protein data bank (PDB)-6LU7 retrieved from Research Collaboratory for

Structural Bioinformatics (RCSB). 3D componential structure of lead molecules and protein were docked using AutoDock analytical tool version 4. Docking simulations were performed using the programmed algorithm inbuilt with pre automation in the software. 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 [12],[13].

3. Results

Docking score implicates the binding affinity between the lead and target higher the negativity in the value that showcase the level of potency of the drug. Development and advancement in the field of computational analysis increased the precision level in identifying the potential drug molecule and deriving its mechanism of action at target site. Selective alterations in the functional groups greatly minimize the non-specific binding and impedes the adverse event at clinical level. Total of 9 bioactive lead compounds were retrieved from the herb *Moringa oleifera*. Out of nine compounds' the lead molecules such as Beta-Sitosterol and Zeatin has maximum of 6 interactions with the core active amino acid residues present on the target. Followed by this the compounds such as Alpha tocopherol, Quercetin and Chlorogenic acid ranked second and with the maximum of 5 and 4 interactions with the active site of the target enzyme 3CLpro.Interaction analysis represented in Figure 3 and 4, Data's were tabulated in Table 2 and 3.

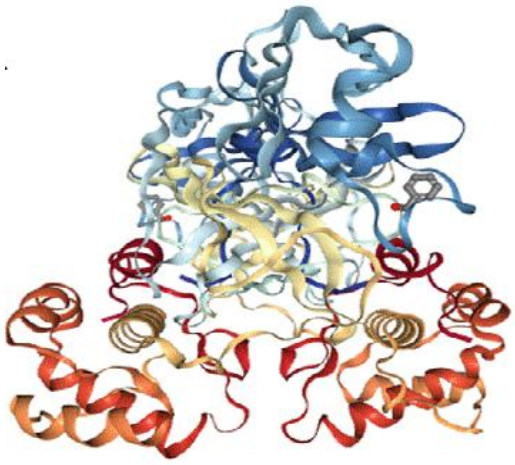

Figure 1: 3D crystalline structure of the target protein COVID-19 main protease (3-chymotrypsin-like protease (3CL pro) – PDB 6LU7

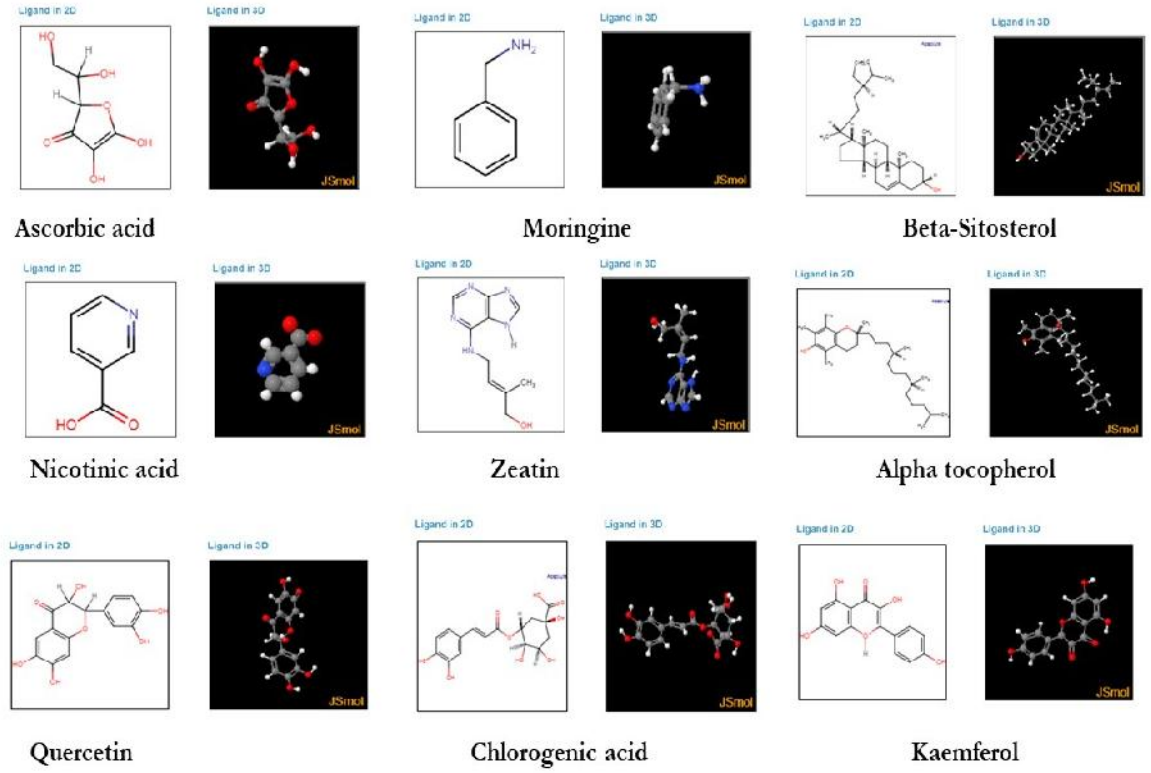

Figure 2: 2D and 3D Structure of Selected Ligands

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
Ascorbic acid	176.12 g/mol	$C_6H_8O_6$	4	6	2
Moringine	311.36 g/mol	$C_{14}H_{17}NO_5S$	3	7	4
Beta-Sitosterol	414.7g/mol	$C_{29}H_{50}O$	1	1	6
Nicotinic acid	123.11 g/mol	$C_6H_5NO_2$	1	3	1
Zeatin	219.24 g/mola	$C_{10}H_{13}N_5O$	3	5	4
Alpha tocopherol	472.7 g/mol	$C_{31}H_{52}O_3$	0	3	14
Quercetin	302.23 g/mol	$C_{15}H_{10}O_7$	5	7	1
Chlorogenic acid	354.31 g/mol	$C_{16}H_{18}O_9$	6	9	5
Kaemferol	286.24 g/mol	$C_{15}H_{10}O_6$	4	6	1

Table 2: Summary of the molecular docking studies of compounds against COVID-19 main protease (3-chymotrypsin-like protease (3CL pro) – PDB 6LU7

Compounds	Binding Free energy Kcal/mol	Inhibition constant Ki µM (*mM)(**nM)	Electrostatic energy Kcal/mol	Intermolecular energy Kcal/mol	Total Interaction Surface
Ascorbic acid	-4.56	456.88	-0.10	-3.52	427.78
Moringine	-5.26	138.91	-1.74	-5.89	328.99
Beta-Sitosterol	-9.53	103.78**	-0.05	-10.89	940.19
Nicotinic acid	-3.74	1.83*	-0.02	-4.03	311.39
Zeatin	-5.70	66.31	-0.01	-5.13	574.30
Alpha tocopherol	-9.11	208.38**	-0.21	-11.63	917.63
Quercetin	-6.80	10.38	-0.17	-6.85	611.20
Chlorogenic acid	-6.61	14.37	-0.08	-7.90	741.79
Kaemferol	-7.29	4.54	-0.11	-7.67	603.66

Table 3: Amino acid Residue Interaction of Lead against COVID-19 main protease (3-chymotrypsin-like protease (3CL pro) – PDB 6LU7

Molecule	Interactions	Amino Acid Residue- Binding									
Ascorbic acid	3	142 ASN	144 SER	145 CYS	163 HIS	165 MET	189 GLN				
Moringine	3	140 PHE	141 LEU	142 ASN	163 HIS	166 GLU	172 HIS				
Beta-Sitosterol	6	27 LEU	41HIS	142 ASN	144 SER	145 CYS	165 MET	166 GLU	168 PRO	189 GLN	
Nicotinic acid	3	140 PHE	142 ASN	144 SER	145 CYS	163 HIS	166 GLU				
Zeatin	6	41 HIS	140 PHE	141 LEU	144 SER	145 CYS	163 HIS	165 MET	166 GLU	172 HIS	189 GLN
Alpha tocopherol	5	41 HIS	59 MET	54 TYR	140 PHE	144 SER	145 CYS	163 HIS	165 MET	166 GLU	189 GLN
Quercetin	5	140 PHE	144 SER	145 CYS	163 HIS	165 MET	166 GLU	189 GLN	192 GLN		
Chlorogenic acid	4	49 MET	142 ASN	145 CYS	163 HIS	165 MET	168 PRO	189 GLN	192 GLN		
Kaemferol	2	41 HIS	49 MET	54 TYR	165 MET	168 PRO	189 GLN	192 GLN			

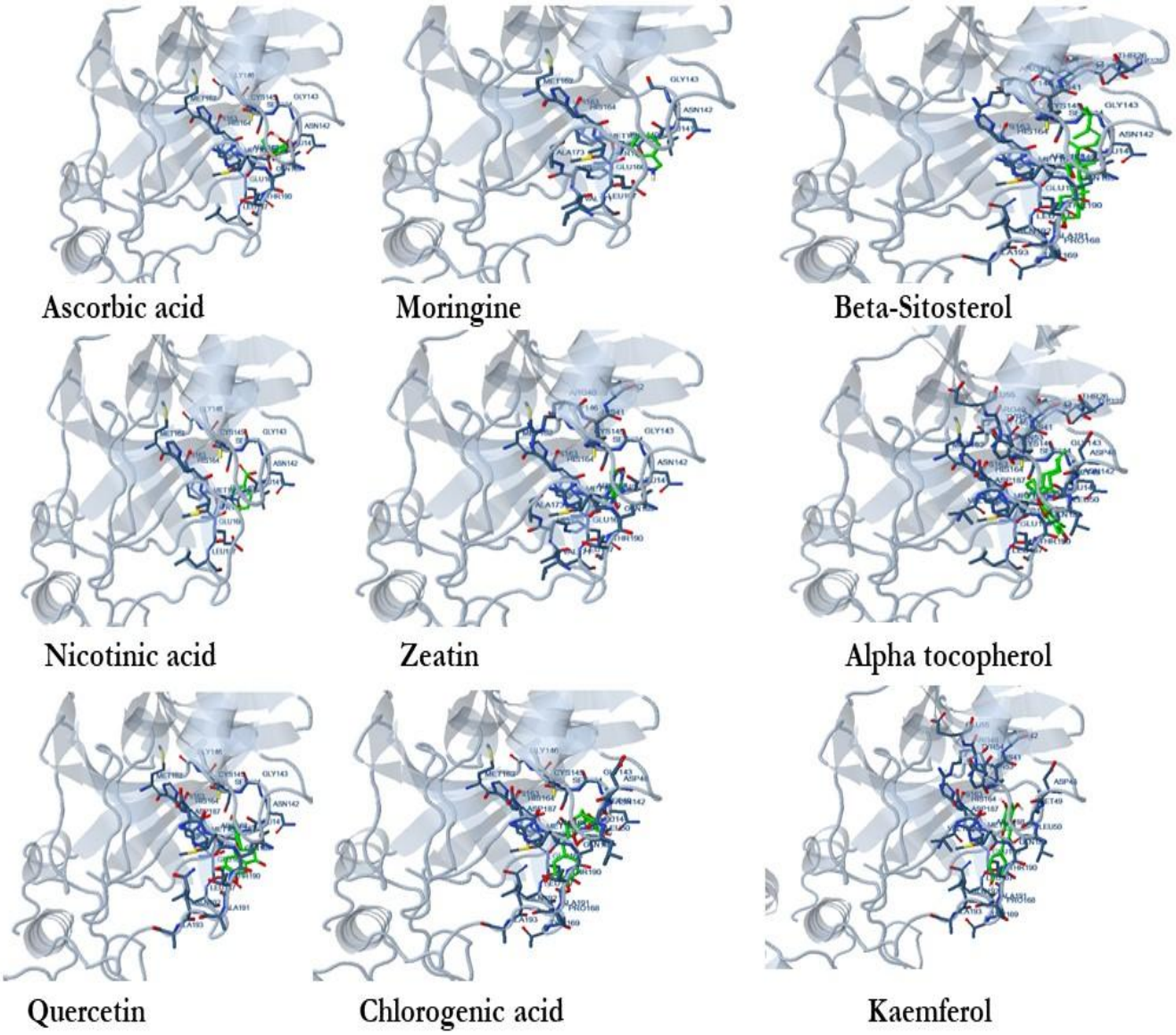

Figure 3: Representing best docking pose of lead molecules against COVID-19 main protease (3-chymotrypsin-like protease (3CL pro)) -PDB- 6LU7

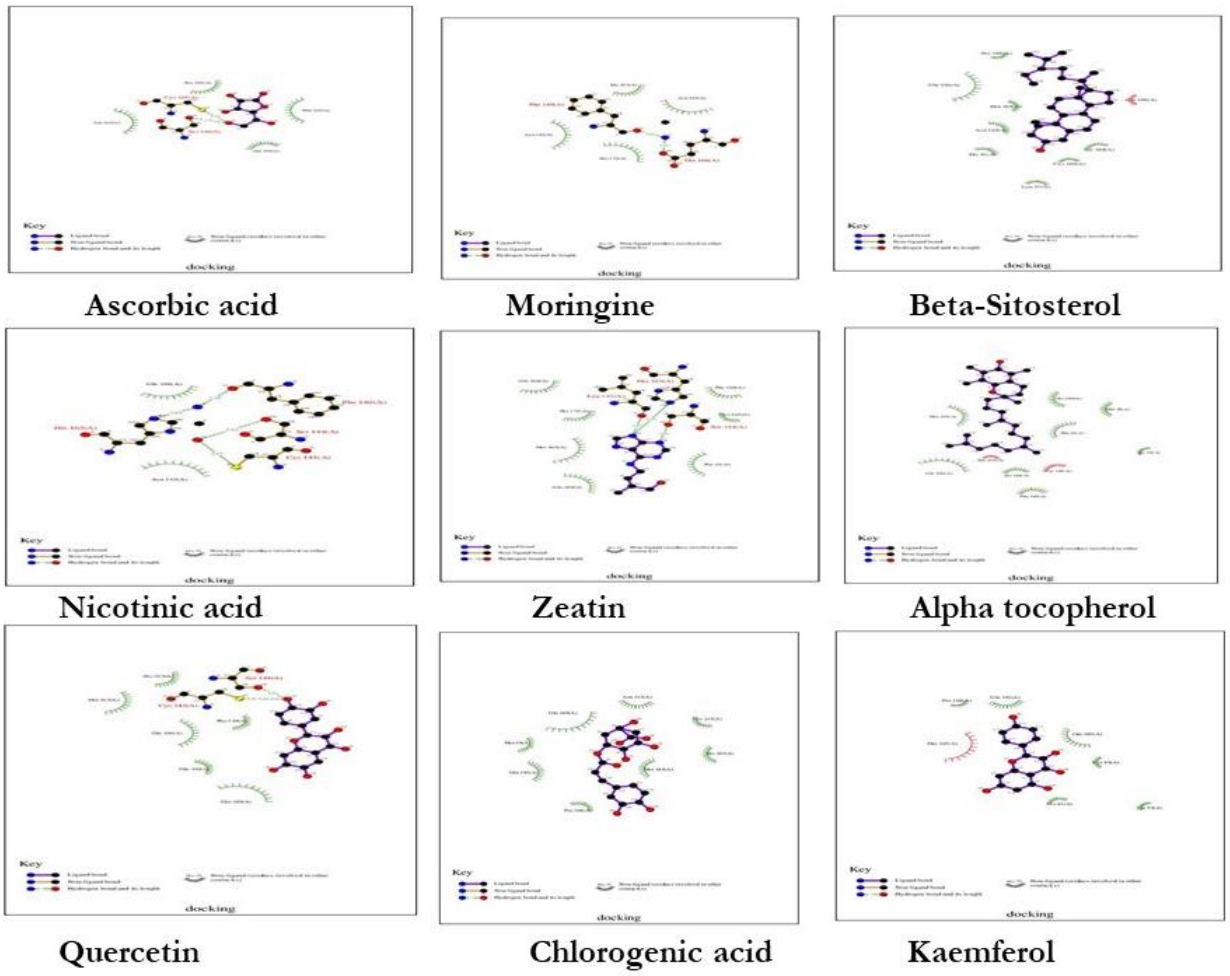

Figure 4: Representing interaction analysis plot of FDA approved lead molecules against COVID-19 main protease (3-chymotrypsin-like protease (3CL pro)) -PDB- 6LU7

4. Discussion

The use of *in silico* approaches as chemoinformatics, molecular modeling, and artificial intelligence (AI) has significantly increased in the last decades [14]. Indeed, in silico approaches now enable the virtual screening of millions of compounds in an affordable time, thus reducing the initial costs of hit identification and improving chances of finding the desired drug candidates.

A natural product (NP) is generally defined as a chemical compound or substance that is produced by living organisms. NPs can be classified by many criteria and characteristics, such as source, biological function, biosynthetic pathway, physical and chemical properties, etc. Nowadays, NPs find a broad spectrum of applications related to human life, including an important role in medicine. Notably, the use of natural products as medicines has been described throughout human history in terms of substances related to herbal medicines, potions, oils, remedies, and foods. Many of these substances have been discovered by trial and error, and through the years they have become standard products in human lives [15].

Molecular docking consists of three main connected goals: pose prediction, virtual screening and binding affinity estimation [16]. A successful docking methodology must be able to correctly predict the native ligand pose within the receptor binding site (i.e. to find the experimental ligand geometry within a certain tolerance limit) and the associated physical–chemical molecular interactions. Furthermore, when investigating large compound libraries, the method must be able to successfully distinguish binding from non-binding molecules and to correctly rank these ligands among the best compounds in the database [17].

In the past, herbal medicine has played an important role in controlling infectious diseases. Clinical evidence from a range of studies of herbal medicine in the treatment of SARS coronavirus (SARS-CoV) has shown significant results, and supported the idea that herbal medicine has a beneficial effect in the treatment and prevention of epidemic diseases [18]. A Cochrane systematic review reported that herbal medicine combined with Western medicine may improve symptoms and quality of life in SARS-CoV patients [19].

In our present investigation total of 9 bioactive lead compounds were retrieved from the herb *Moringa oleifera*. Out of nine compounds' the lead molecules such as Beta-Sitosterol and Zeatin has maximum of 6 interactions with the core active amino acid residues present on the target. Followed by this the compounds such as Alpha tocopherol, Quercetin and Chlorogenic acid ranked second and with the maximum of 5 and 4 interactions with the active site of the target enzyme 3CLpro.

5. Conclusion

The enzyme $3CL^{pro}$ is considered to be a promising drug target and a lot of efforts have been committed to its study because of its key role in the replication cycle of the virus. Nature provides a vast library of chemicals to explore and develop drugs for treatment of various ailments including viral diseases. To date, a good number of herbal medicines or their constituents have shown potential antiviral activity. It was concluded from the outcome of the present investigation that phytochemical present in the herb Moringaoleifera offers potential binding affinity with the enzyme $3CL^{pro}$ thereby it may be expected to halt the replication and progression of virus upon proper clinical recommendation. Hence, Moringaoleifera can be the better choice to treat Covid-19.It will be useful in this Covid-19 pandemic situation and in future it have tobe used in numerous siddha formulations.

Acknowledgments

We wish to acknowledge our thanks The Director, National Institute of Siddha, Chennai, Tamil Nadu.The Tamil Nadu Dr. M.G.R. Medical University, Chennai, India and to The Noble research solutions, Chennai, Tamil Nadu, India for their timely and valuable support.

6. References

1. Huang C., Wang Y., Li X., Ren L., Zhao J., Hu Y. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. Lancet. 2020; 395(10223):497–506.
2. Anand K., Ziebuhr J., Wadhwani P. Coronavirus main proteinase (3CLpro) structure: basis for design of anti-SARS drugs. Science. 2003; 300:1763–1767.
3. Needle D., Lountos G.T., Waugh D.S. Structures of the middle east respiratory syndrome coronavirus 3C-like protease reveal insights into substrate specificity. Acta Crystallogr. D Biol. Crystallogr. 2015; 71: 1102–1111.
4. Ghosh A.K., Xi K., Ratia K. Design and synthesis of peptidomimetic severe acute respiratory syndrome chymotrypsin-like protease inhibitors. J. Med. Chem. 2005; 48: 6767–6771.
5. Mishra B.B., Tiwari V.K. Natural products: An evolving role in future drug discovery. Eur. J. Med. Chem. 2011; 46: 4769–4807.
6. Cragg G.M., Newman D.J. Biodiversity: A continuing source of novel drug leads. Pure Appl. Chem. 2005; 77: 7–24.
7. Cragg G.M., Newman D.J. Natural products: A continuing source of novel drug leads. Biochim. Biophys. Acta. 2013; 1830: 3670–3695.
8. Dohlman H.G. Thematic Mini review Series: New Directions in G Protein-coupled Receptor Pharmacology. J. Biol. Chem. 2015; 290:19469–19470.
9. Gloriam D.E., Fredriksson R., Schiöth H.B. The G protein-coupled receptor subset of the rat genome. BMC Genom. 2007; 8:338.
10. Soppa J. Two hypotheses—one answer. Sequence comparison does not support an evolutionary link between halobacterial retinal proteins including bacteriorhodopsin and eukaryotic G-protein-coupled receptors. FEBS Lett. 1994; 342:7–11.
11. Stefano,F; Ruth, H.et al. Computational protein-ligand docking and virtual drug screening with the AutoDock suite. Nat Protoc. 2016, 11, 905–919.
12. Williams et al. MolProbity: More and better reference data for improved all-atom structure validation. Protein Science.2018, 27, 293-315.
13. Morris GM. Automated docking using a Lamarckian genetic algorithm and an empirical binding free energy function. Journal of Computational Chemistry.1998, 19, 1639-1662.

14. Song C.M., Lim S.J., Tong J.C. Recent advances in computer-aided drug design. Brief. Bioinform. 2009; 10:579–591.
15. Dias D.A., Urban S., Roessner U. A Historical Overview of Natural Products in Drug Discovery. Metabolites. 2012; 2:303–336.
16. Jain AN, Nicholls A. Recommendations for evaluation of computational methods. J Comput Aided Mol Des. 2008; 22:133–139.
17. Kolb P, Irwin JJ. Docking screens: right for the right reasons? Curr Top Med Chem. 2009; 9: 755–770.
18. Nigeria Centre for Disease Control (NCDC) Updates on COVID-19 (Coronavirus Disease 2019): case summary in Nigeria as at April 20th 2020.
19. Vincent M J., Bergeron E., Benjannet S. et al.. Chloroquine is a potent inhibitor of SARS coronavirus infection and spread. Virol J. 2005; 2:69.

Access this Article in Online

Website: www.darshanpublishers.com

Subject: Siddha Medicine

Quick Response Code

How to cite this article:
M. K. Sathesh Kumar, P. Sharmila, P. Jayapriya, T. Lakshmikantham, R. Meenakumari. (2020). *In-Silico* Evaluation of Anti-Viral Potential of Siddha herbal Formulation *Moringa oleifera* (Murungai) against 3-CLpro Enzyme target in the treatment of SARS Co-V-2 (COVID-19). Int. J. Curr. Res. Biol. Med. 5(11): 1-9.
DOI: http://dx.doi.org/10.22192/ijcrbm.2020.05.11.001