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In Silico Analysis of Sponges Compound Against Mpro COVID-19: A Review

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Abstract. The novel coronavirus 2019 (COVID-19) is one of the viruses that can infect humans and cause high mortality worldwide. The protease (Mpro) is a key 2019-nCoV enzyme mediate viral replication and transcription. Mpro is currently used as the candidate for the COVID-19 vaccine but yet the interaction of the protein is to be determined. The Mpro can be a target protein to design the drug of COVID-19. The drug design from natural products that are considered to have low toxicity is needed against the virus. The aim of this study to determine the potential of some marine compounds against COVID-19 in silico and to find the potential amino acid residues between interaction ligand-protein receptors. The methods of this study use virtual screening of Autodock Vina and study literature to find the action mechanism between ligand and protein receptors. The result of this study was the selected marine compound which may have the potential for 2019-nCoV to be future drugs. The interaction between the best ligand-protein predominant by glutamine and threonine amino acid. The role of glutamine as immune nutrition for recovery patients for some disease and maintain the body back to the homeostasis. Threonine could contribute to inflammatory responses against foreign substances.

Keywords: COVID-19, in silico, Mpro, sponges

Running title: In Silico of Sponges and Mpro COVID-19

INTRODUCTION

Coronaviruses are a group of viruses that generally affect the respiratory system of mammals, and can cause acute respiratory infections. The new strain of coronaviruses known as SARS-CoV-2 was originated from Wuhan city, Hubei province of China, in late December 2019 and then later spread across the entire globe (Adhikari et al., 2020).

Based on report of WHO, in 5 October 2020, there have been 35,109,317 confirmed cases of COVID-19, including 1,035,341 deaths. In America 17,101,686 confirmed cases leading worldwide and South east asia reach up 7,418,537 confirmed case. In Indonesia, from Jan 3 to 5 October 2020, there have been 303,498 confirmed cases of COVID-19 with 11,151 deaths.

The characteristics of Coronaviruses (CoVs) are enveloped, positive-sense and single-stranded RNA (Cui et al., 2019). They were classified into three main groups based on their antigenic properties: 1) alpha-CoVs that responsible for gastrointestinal disorders, 2) beta-CoVs divided into three types : Bat coronavirus (BCoV), human Severe Acute Respiratory Syndrome (SARS) virus, and Middle Eastern Respiratory Syndrome (MERS) virus; and 3) gamma-CoVs that infect avian species (Schoeman and Fielding, 2019). We mostly familiar with the SARS-CoV responsible for an outbreak in 2002-2003 (Peiris et al. 2004) and MERS-CoV in 2012 (Zaki et al. 2012).

The main protease (Mpro), also known as a chymotrypsin-like cysteine protease, 3CLpro) is one of the key enzymes in the viral life cycle that essential for interactions between the virus and host cell receptor during viral entry (Zumla et al. 2016). Mpro are highly conserved, sharing more than 90% sequence similarity with the corresponding SARS-CoV enzymes (Liu et al. 2020).

Mpro is characterized by a self-cleavage protein and consists of a homodimer subdivided into two protomers (A and B) that have three distinct domains (Cui et al., 2019; Kannan et al. 2020). The first and second domains have antiparallel structure of β sheets while the third domain contains five α helices forming a globular group (Jin et al. 2020). Mpro has been found to play a fundamental role in viral gene expression and replication. Therefore, this is the reason Mpro is an attractive candidate target for anti-CoV drug design.

The mechanism action of antiviral therapy divided in three approaches for control the viral infections: (a) vaccination, (b) stimulation of host resistance mechanisms, and (c) antiviral chemotherapy. Antivirals are drugs that inhibit certain virus-specific events, such as binding to host cells, which is how SARS-Cov-2 binds to ACE2 (Hoffamann et al. 2020) (Zumla et al. 2016). Most antiviral drugs are targeted to nucleic acids in viruses. The main problems of viral therapy was to find a drug that specific to fight the virus. Antivirals are frequently more effective in prevention than in the treatment itself, and are ineffective in eliminating latent or nonreplicating viruses (Crumpacker, 2004). In addition, when selecting an antiviral drug, viral resistance must also be considered since it is one of the main causes of therapeutic failure.

However, no effective antiviral treatment or vaccine is available for COVID-19 until now. Presently the SARS-CoV-2 infected patient's treatments have been limited to the use of prophylactic and symptomatic regulation like mild symptoms such as dry cough, sore throat, and fever, and various fatal complications (Chen et al. 2020). Therefore, there is an urgent need for the discovery of a potential treatment therapy and novel drugs from natural product to get effectivity and minimal toxicity against the SARS-CoV-2 (Cui et al. 2019).

Natural compounds can be obtained from several plants, microorganisms, and marine organisms (Natalia et al.,

2017). Some secondary metabolites can derived from sponge. In this study, we present selected natural product as inhibitor of Mpro in COVID-19 like Esculetin ethyl ester, Hamigeran B, Polyacetylenetriol and Dehydrofurodendin.

Esculetin ethyl ester isolated from *Axinella cf. corrugata* sponges that have activity to inhibit recombinant 3CLpro in vitro. It has also been shown that an effective inhibitor of SARS-CoV replication in Vero cells and that it mediates these effects at non-cytotoxic concentrations (Lira et al, 2007). Polyacetylenetriol was isolated from *Petrosia* sp. that have activity inhibition of HIV-1 reverse transcriptase. This compound inhibits the RNA- and DNA-dependent DNA polymerase activities of retroviral reverse transcriptases (RTs) (Loya et al. 2002). The dehydrofurodendin were isolated from the sponge *Lendenfeldia* sp. collected at Madagascar. This compound can inhibit RT RNA and DNA-directed DNA polymerase (Chill et al. 2004). Hamigeran B was isolated from the marine sponge *Hamigera tarangaensis* (family Anchinoidae) from the Hen and Chicken Islands in New Zealand and showed 100% *in vitro* virus inhibition against both the herpes and polio virus but little cytotoxicity (Muller et al. 2000; Wellington et al. 2000; Clive and Jian Wang, 2003). In this paper, we selected this marine compound from sponge because this compounds have potential activity as antiviral.

Molecular docking is an attractive tools to find out the novel drug design and discovery, as well as in the mechanistic study by a molecule target (ligand) into the binding site of the target specific of the DNA/protein (receptor) with a stable interaction, potential efficacy and more specificity (Rohs et al. 2005; Guedes et al. 2014). The molecular docking approach can be used to model the interaction between a small molecule and a protein at the atomic level with the similarity result reach up 90% like in vitro result (McConkey et al. 2002). The basic docking process involves prediction of the ligand conformation with active site and assessment of the binding affinity (Xuan Yu Meng et al. 2011).

The aim of this study was to find out the potential of selected compound from marine sponge as antiviral drug and analysis the amino acid interaction between marine sponge compound and Mpro of COVID-19. In this study, we analyze the potential of selected compound marine sponge for the novel drugs discovery against the corona virus. From this information, we then established the specifity potent compound as antiviral.

MATERIALS AND METHODS

In silico study of the target marine sponge compound with M^{pro}

The in silico test was carried out using 2019-nCoV main protease M^{pro}. The 3D structure is obtained from the protein data bank (www.pdb.org) using the PDB code. The 3D structure of the target marine sponge compound was obtain from Pubchem (https://pubchem.ncbi.nlm.nih.gov) and converted by open babel. The energy affinity of the marine sponge compound and M^{pro} target protein was carried out with Autodock Vina. The lowest energy affinity is chosen to determine the most potent drug candidate. The interaction between ligands and amino acid residues on the active site was carried out using Argus Lab. Furthermore, the study of M^{pro} that bind to the marine sponge compound was carried out to determine the mechanism of action of the drug candidate. Finally, the protein structure was minimized to Root Mean Square Deviation (RMSD) value with score <2. Thus, analysis of interaction ligand and protein target was done to determine amino acid residues that interact between ligand and protein.

RESULTS AND DISCUSSION

A novel nowadays emerged SARS-CoV-2 is presenting major threats to human health over the world (Zhu N et al. 2020). Currently, no specific clinical therapeutics and vaccine are available for the treatment of SARS-CoV-2-mediated infections (Zhou Yet al. 2020). Thus, there is an urgent need to identify and characterize novel drug candidates to overcome the health losses caused by SARS-CoV-2. To provide natural scaffolds for drug development, we have selected some marine compound from sponge against novel drug target, M^{pro}.

The antiviral compounds are currently of particular interest since viral diseases have become major human health problems in recent decades (Sagar et al. 2010; Nannou et al. 2020). The ability of a virus to rapidly evolve and develop resistance to existing pharmaceuticals calls for continuing development of new antiviral drugs (Rabaan et al. 2020). Several lead antiviral compounds have been isolated from marine natural resources and there has been a consistent effort to identify new compounds. Based on the above rational, this study focus on existing or promising antiviral lead compounds such as Esculetin ethyl ester, Polyacetylenetriol, Dehydrofurodendin, and Hamigeran B from marine resources (Sagar et al. 2010; Uzair et al. 2011) which may have the potential for 2019-nCoV to be future drugs.

Bioinformatics is one of the most essential and straight forward approaches to design new drugs (Lin et al. 2020). Bioinformatics techniques nowadays is very useful because cost effective and easy to use. Due to the high cost of clinical and laboratory trials, the time consuming and the possibility of error the bioinformatics techniques are used to design novel drug potential (Shaghaghi, 2020). Computational docking can be used to predict the conformations and energy affinity of binding for small molecule ligands to protein targets. Docking is widely used for the study of biomolecular interactions and applied to structure based drug design (Vijayaraj et al. 2019). The dock scoring function can be used to determine the binding mode and site of a ligand, predict binding affinity and identify the potential drug. Several studies have been conducted to discover 2019-nCoV antiviral drugs (Jin et al.

2020; Sampangi-Ramaiah et al. 2020). The results of some studies shown that protease inhibitors which major part of secondary metabolites derivatives, can be effective to controll viral infection.

The results of virtual screening of target marine sponge compound against M^{pro} were determined. The bond energies have been tested by RMSD and compared with crystallographic ligands. marine sponge compound as drug candidates can be done quickly and easily with the help of molecular docking. Virtual screening of drug candidates from natural ingredients helps to discover new molecules and the activity of the targeted compounds.

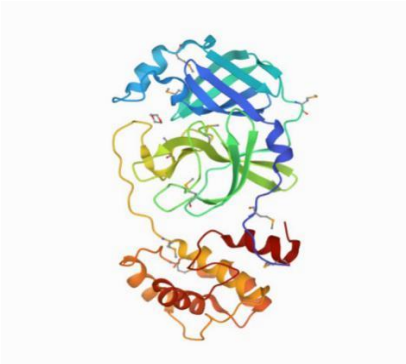

Figure 1. Coronavirus Main Proteinase (3CLpro) Structure (pdb.org).

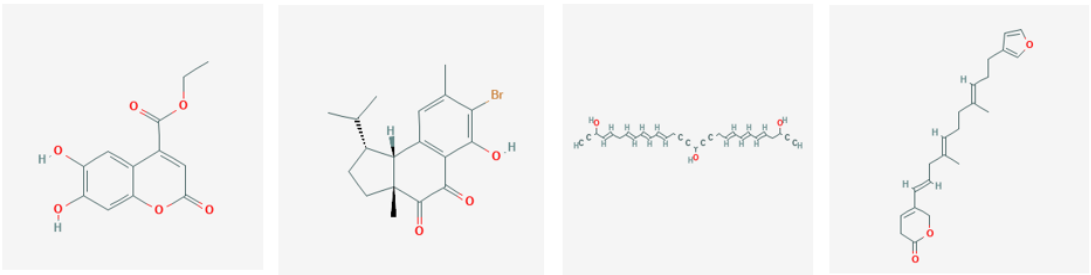

Figure 2. a. Esculetin ethyl ester b. Hamigeran B, c. Polyacetylenetriol and d. dehydrofurodendin

Table 1. Docking score marine sponge compound against M^{pro}

Ligand	M^{pro} (N3) kcal/mol
Esculetin ethyl ester	-8.42
Polyacetylenetriol	-7.4
Dehydrofurodendin	-6.28
Hamigeran B	-5.798

Docking has been successfully performed between selected marine sponge compound with M^{pro}. The docking results show that esculetin ethyl ester is the most potent compound among other ligand. This is evidenced by the lowest energy affinity value produced on esculetin ethyl ester with a score of -8.42 when it binds to M^{pro}. Based on this study, we can determine the best energy affinity esculetin ethyl ester > polyacetylenetriol > dehydrofurodendin > hamigeran B.

The esculetin ethyl esther is classified as polyketide and can inhibit SARS-Corona virus viral protease 3CL inhibition but the mechanism still undetermined (Lira, 2007). Polyacetylenetriol included Fatty acid that can inhibit RNA- and DNA-directed DNA polymerase and Reversible non-competitive inhibition, with hydrophobic interaction (Loya et al. 2002). Dehydrofurodendin includes of furanoterpene compound that inhibit RT RNA and DNA-directed DNA polymerase, but the mechanism unclear (Chill et al. 2004). Hamigeran B is included as phenolic macrolides (Sagar et al. 2010; Zappe et al. 2008) shows 100% in vitro inhibition against both the herpes and polio viruses (Wellington et al. 2000).

The several reports shows that esculetin ethyl esther activity as an antiinflammatory agent. Esculetin reduces the organ tissue damage from inflammation. Thus, esculetin inhibits the secretion of soluble intercellular adhesion molecule (sICAM-1), which can reduce the adhesion reaction of leukocytes as well as endothelial cells in order to reduce inflammation (Duan et al. 2007).

The docking poses of all the ligands were visualized using Argus Lab. Four ligands with the highest binding affinity to M^{pro} were visualized and the binding residues of Mpro in the binding pocket were analyzed. Esculetin ethyl ester was interacting with Gln 192 and Thr 190 residues in the binding pocket of M^{pro}. The residues Glu166, Gly143, Gln 192 and Arg 188 of binding pocket were responsible for the binding of Polyacetylenetriol in the binding pocket. Dehydrofurodendin showed interaction with Gly 143 and Ser 144 residues of M^{pro}. Hamigeran B showed the Ser 144 and His 163.

The interaction amino acid between esculetin ethyl ester and M^{pro} were glutamin and threonin. Glutamine, which can modulate and preserve lung function. Glutamine also reduce the production of pro-inflammatory cytokines (Aquilani et al. 2015;. Aquilani et al. 2014). Cytokine-

mediated effects are an essential part response to infection but excessive production of pro-inflammatory cytokines increases the risk of pneumonia and death in COVID-19 patients. Some research reported that amino acids have anti-inflammatory effects. The integration of arginine, glutamine, or glycine decrease lung damage caused infections and inflammatory. The existence of glutamine has been shown to significantly reduce inflammatory cytokines without affecting other parameters. Arginine or glycine supplementation may be a new nutritional strategy to reduce the bacterial infection on alveolar function (Solerte et al. 2008; Buondonno et al. 2020). Based on the other research, threonine compounds could contribute to inflammatory responses against foreign substances and may inhibit the M^{pro} of SARS-CoV-2 (Chen et al. 2017) but the action mechanism of this amino acid still unclear.

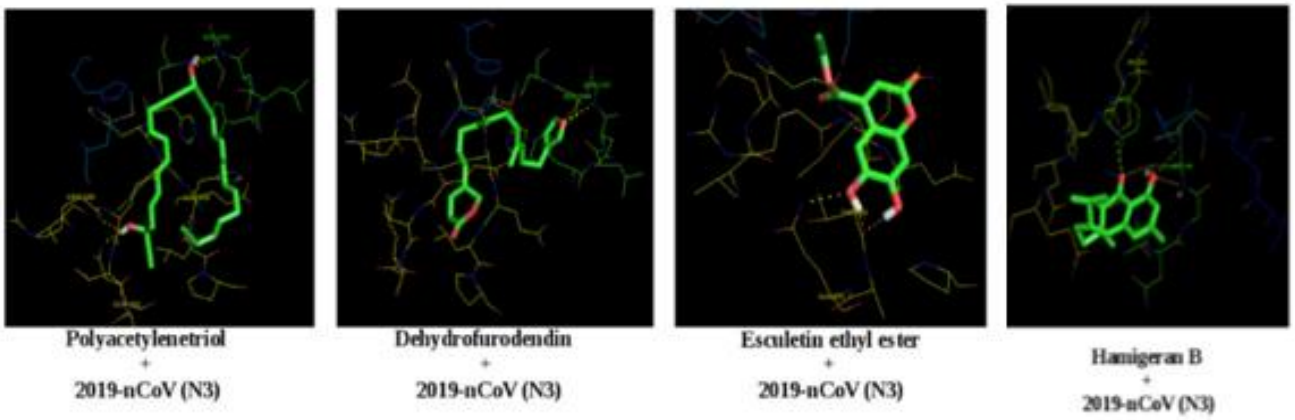

Figure 3. Visualization of interaction amino acid between selected marine compound against M^{pro}

CONCLUSIONS

In summary, the selected marine compound from sponge can be potential of inhibitor Mpro SARS-CoV-2 to develop with another vaccine. The selected marine compound from sponge have already been successfully docked against viral disease. Esculetin ethyl ester from *Axinella cf. corrugate* has potential energy affinity -8.42. The amino acid residues between Esculetin ethyl ester and M^{pro} were glutamin and threonine, can be important amino acid that can bind and interact with M^{pro}. Glutamine is very important in immune nutrition and recovery. Threonine may inhibit the Mpro of SARS-CoV-2 but the action mechanism of this amino acid still unclear.

The benefit of this study is the result and analysis of docking can used to obtain the potential of sponge marine compound as novel drug as inhibitor of M^{pro} COVID-19. This result can continue to study the effectivity of selected marine compound with in vivo and in vitro research. The in silico technique can be used to study of the potential of novel compound as anticancer, antitumor and antiinflamation.

In the present study may prove valuable for exploring and developing novel natural anti-COVID-19 therapeutic agents in the future. In addition, a multidisciplinary approach (organic chemistry, biochemistry, molecular biology, and molecular genetics) is needed to develop potential of natural product as anticancer drug discovery.

REFERENCES

Adhikari, S. P., Meng, S., Wu, Y.-J., Mao, Y.-P., Ye, R.-X., Wang, Q.-Z., Sun, C., Sylvia, S., Rozelle, S., Raat, H., &

Zhou, H. 2020. Epidemiology, causes, clinical manifestation and diagnosis, prevention and control of coronavirus disease (COVID-19) during the early outbreak period: A scoping review. Infectious Diseases of Poverty 9(1), 29. 16(1):69.

Aquilani R, Boselli M, D'Antona G, et al. 2014. Unaffected arm muscle hypercatabolism in dysphagic subacute stroke patients: the effects of essential amino acid supplementation. Biomed Res Int.

Aquilani R, Emilio B, Dossena M, Baiardi P, Testa A, Boschi F, Viglio S, Iadarola P, Pasini E, Verri M. 2015. Correlation of deglutition in subacute ischemic stroke patients with peripheral blood adaptive immunity: essential amino acid improvement. Int J Immunopathol Pharmacol 28(4):576–583.

Buondonno I, Sassi F, Carignano G, Dutto F, Ferreri C, Pili FG, Massaia M, Nisoli E, Ruocco C, Porrino P, Ravetta C, Riganti C, Isaia GC, D'Amelio P. 2020. From mitochondria to healthy aging: the role of branched-chain amino acids treatment: MATeR a randomized study. Clin Nutr. 39:2080–2091.

Chen N, Zhou M, Dong X, Qu J, Gong F, Han Y, et al. 2020. Epidemiological and clinical characteristics of 99 cases of 2019 novel coronavirus pneumonia in Wuhan, China: a descriptive study. Lancet ;395:507–13

Chen YP, Cheng YF, Li XH, Yang WL, Wen C, Zhuang S, Zhou YM. 2017. Effects of threonine supplementation on the growth performance, immunity, oxidative status, intestinal integrity, and barrier function of broilers at the early age. Poult Sci.

Chill L, Rudi A, Aknin M, Loya S, Hizi A, Kashman Y, et al. 2004. New sesterterpenes from Madagascan Lendenfeldia sponges. Tetrahedron, 60, 10619-10626.

Clive D and Jian Wang. 2003. Stereospecific Total Synthesis of the Antiviral Agent Hamigeran B—Use of Large Silyl Groups to Enforce Facial Selectivity and to Suppress Hydrogenolysis. Angew. Chem. Int. Ed. 2003, 42, 3406 – 3409.

Crumpacker CS. 2004. Use of antiviral drugs to prevent herpes virus transmission. New England Journal of Medicine 350:67-68

Cui J, Li F, Shi Z-L. 2019. Origin and evolution of pathogenic coronaviruses. Nat Rev Microbiol ;17:181–92.

Duan H.Q., Zhang Y.D., Fan K., Suo Z.W., Hu G., Mu X. 2007. Anti-inflammatory mechanism of esculetin. Chin. J. Vet. Med 43:45–46

Guedes IA, de Magalhães CS, Dardenne LE. 2014. Receptor-ligand molecular docking. Biophysical Reviews 6: 75-87.

Hoffmann M, Kleine-Weber H, Schroeder S, Krüger N, Herrler T, Erichsen S, Schiergens TS, Herrler G, Wu NH, Nitsche A, Müller MA, Drosten C, Pöhlmann S. 2020. SARS-CoV-2 Cell Entry Depends on ACE2 and TMPRSS2 and Is Blocked by a Clinically Proven Protease Inhibitor. Cell.

Jin, Z., Du, X., Xu, Y. et al. Structure of Mpro from COVID-19 virus and discovery of its inhibitors. 2020. Nature Kannan S, Shaik Syed Ali P, Sheeza A, Hemalatha K. 2020. COVID-19 (Novel Coronavirus 2019)– recent trends. European Review for Medical and Pharmacological Sciences 24:2006-2011.

Lin X, Li X, Lin X. 2020. A Review on Applications of Computational Methods in Drug Screening and Design. Molecules.

Lira Simone P, Mirna H. R. Seleghim,a David E. Williams,b Frederic Marion,b Pamela Hamill,c François Jean,c Raymond J. Andersen,b Eduardo Hajdud and Roberto G. S. Berlinck. 2007. A SARS-Coronovirus 3CL Protease Inhibitor Isolated from the Marine Sponge Axinella cf. corrugata: Structure Elucidation and Synthesis. J. Braz. Chem. Soc., Vol. 18, No. 2, 440-443.

Liu W., Morse J.S., Lalonde, T. Xu S. 2020.Learning from the Past: Possible Urgent Prevention and Treatment Options for Severe Acute Respiratory Infections Caused by 2019-nCoV. ChemBioChem 21, 730–738.

Loya S, Amira Rudi, Yoel Kashman, and Amnon Hizi. 2002. Mode of inhibition of HIV-1 reverse transcriptase by polyacetylenetriol, a novel inhibitor of RNA- and DNA-directed DNA polymerases. Biochem J15; 362(Pt 3): 685–692.

McConkey BJ, Sobolev V, Edelman M. 2002. The performance of current methods in ligand-protein docking. Current Science 83:845–855.

Muller W, Bohm M, Batel R, De Rosa S, Tommonaro G, Muller I, Schroder H. 2000. Application of cell culture for the production of bioactive compounds from sponges: synthesis of avarol by primmorphs from Dysidea avara. J. Nat. Prod 63:1077–1081.

Nannou C, Ofrydopoulou A, Evgenidou E, Heath D, Heath E, Lambropoulou D. 2020. Antiviral drugs in aquatic environment and wastewater treatment plants: A review on occurrence, fate, removal and ecotoxicity. Sci Total Environ 699:134322.

Natalia Bailon-Moscoso, Gabriela Cevallos-Solorzano, Juan Carlos Romero-Benavides, and Maria Isabel Ramirez Orellana. 2017. Natural Compounds as Modulators of Cell Cycle Arrest: Application for Anticancer Chemotherapies Current Genomics 18, 106-131.

Peiris JS, Guan Y, Yuen KY. 2004. Severe acute respiratory syndrome. Nature Medicine 10(12 Suppl):S88-97.

Rabaan AA, Al-Ahmed SH, Sah R, Tiwari R, Yatoo MI, Patel SK, Pathak M, Malik YS, Dhama K, Singh KP, Bonilla-Aldana DK. 2020. SARS-CoV-2/COVID-19 and advances in developing potential therapeutics and vaccines to counter this emerging pandemic virus–a review. Preprints, 2020040075.recent trends. European Review for Medical and Pharmacological Sciences 24:2006-2011.

Rohs R, Bloch I, Sklenar H, Shakked Z. 2005. Molecular flexibility in ab-initio drug docking to DNA: binding-site and binding-mode transitions in all-atom Monte Carlo simulations. Nucl Acids Res 33: 7048-7057.

Sagar, S., Kaur, M., and Minneman, K. P. 2010. Antiviral lead compounds from marine sponges. Mar. Drugs 8, 2619–2638.

Sampangi-Ramaiah MH, Vishwakarma R, Shaanker RU. 2020. Molecular docking analysis of selected natural products from plants for inhibition of SARS-CoV-2 main protease. Curr Sci. 118(7):1087. Shaghaghi N. Molecular Docking study of novel COVID-19 Protease with low risk Terpenoides Compounds of Plants. ChemRxiv.

Schoeman D, Fielding BC. 2019. Coronavirus envelope protein: current knowledge. Virology Journal

Solerte SB, Gazzaruso C, Bonacasa R, Rondanelli M, Zamboni M, Basso C, Locatelli E, Schifino N, Giustina A, Fioravanti M. 2008. Nutritional supplements with oral amino acid mixtures increases whole-body lean mass and insulin sensitivity in elderly subjects with sarcopenia. Am J Cardiol 101(11A):69E–77E

Uzair B, Mahmood Z, Tabassum S. 2011. Antiviral activity of natural products extracted from marine organisms. BioImpacts: BI. 1(4):203

Vijayaraj, R., Altaff, K., Rosita, A. S., Ramadevi, S., & Revathy, J. 2020. Bioactive compounds from marine resources against novel corona virus (2019-nCoV): in silico study for corona viral drug. Natural Product Research, 1–5.

Wellington K.D., Cambie R.C., Rutledge P.S., Bergquist P.R. 2000. Chemistry of sponges, 19: novel bioactive metabolites from Hamigera tarangaensis. J. Nat. Prod. 63:79–85.

World Health Organization. 2020. WHO Coronavirus Disease (COVID-19). World. Health Organization.

Xuan-Yu Meng Hong-Xing Zhang, Mihaly Mezei, and Meng Cui..2011. Molecular Docking: A powerful approach for structure-based drug discovery. Curr Comput Aided Drug Des 1; 7(2): 146–157.

Zaki A.M., van Boheemen S., Bestebroer T.M. 2012. Isolation of a novel coronavirus from a man with pneumonia in Saudi Arabia. The New England Journal of Medicine 367:1814–1820.

Zappe A, M.E. Snell B., M.J. Bossard. 2008. PEGylation of cyanovirin–N, an entry inhibitor of HIV. Advanced Drug Delivery Reviews 60 79–87.

Zhou Y, Hou Y, Shen J, Huang Y, Martin W, Cheng F. 2020. Network-based drug repurposing for novel coronavirus 2019-nCoV/SARS-CoV-2. Cell Discovery.

Zhu N, Zhang D, Wang W, Li X, Yang B, Song J, Zhao X, Huang B, Shi W, Lu R, Niu P, Zhan F, Ma X, Wang D, Xu W, Wu G, Gao GF, Tan W. 2020. A Novel Coronavirus from Patients with Pneumonia in China, 2019. N Engl J Med. 382, 727-733.

Zumla, A.; Chan, J.F.W.; Azhar, E.I.; Hui, D.S.C.; Yuen, K.-Y. 2016. Coronaviruses—Drug discovery and therapeutic options. Nat. Rev. Drug Discov. 15, 327–347.