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Molecular mechanism of virgin coconut oil as a Nsp-3 inhibitor of SARS-CoV-2

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Abstract. Virgin coconut oil (VCO) is a natural product that contains mostly medium-chain lipids, such as palmitates, stearates, and oleates. This study aims to explore whether VCO would make an effective to Nsp3b as one of target for virtual ligand screening of potential drug targets inhibitor of SARS-CoV-2, especially medium-chain content. In this study, computational investigations (in silico) were conducted using five long-chain molecules constituting VCO, namely palmitate, myristate, stearate, laurate, and oleate. Molecular docking simulation was conducted using the PLANTS 1.1. The binding affinity results revealed palmitate, and stearate have lower score than the co-crystalize ligand of Nsp3. Stearate and palmitate binding affinity score were -6.45 and -6.23 respectively, while co-crystalize ligand as our ligand control is -5.71, despite co-crystalize ligand hydrogen bonds is more than both of palmitate and stearate. In addition to molecular docking, we perform molecular dynamic simulation and found stearate relatively stable to bind Nsp3. The RMSD of complex protein to stearate was stable below 1 nm over 20 ns simulation. This could be caused by hydrogen bonds between stearate and Nsp3 protein, where average of hydrogen bond is 1.2, and recorded to be higher during the last 10 ns with an average of 1.5. Both palmitate and stearate also found have biological activity against several virus including adenovirus, poxvirus, and influenza virus with score greater than 0.5 (score from 0 to 1).

Keywords: molecular docking, molecular dynamics, VCO, SARS-CoV-2 inhibitor,

INTRODUCTION

Coronavirus disease 2019 (COVID-19), caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), has globally affected almost all countries (Lanham-New *et al.,* 2003; Zimmerman *et al.,* 2021). It causes fever, fatigue, dry cough, muscle aches, shortness of breath, and in some instances, it leads to pneumonia (Rothan & Byrareddy, 2020). Despite the implementation of strict health protocols to prevent the spread of the disease, the number of patients is still high in countries such as the United States, India, Brazil, and so forth (*COVID Live - Coronavirus Statistics - Worldometer*, 2021). Until now, drugs and vaccines for COVID-19 are still being developed and evaluated.

The undiscovered remedies for COVID-19 triggered all countries to study and observe the potential of medical science to overcome the COVID-19 pandemic. Indonesia has the potential for abundant natural resources, both on land and in the ocean, whose benefits have not been studied yet. Virgin coconut oil (VCO) is a product

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commonly consumed by Indonesians. Until now, to the best of our knowledge, the potential of VCO has not been explored, especially its benefits regarding health and improving the immunity against COVID-19. The content of VCO is mostly long-chain lipids, with the largest proportion being laureate (53.6%), myristate (18.8%), palmitate (10.7%), and oleate (4.6%) (Ströher *et al.*, 2020). Multiple long chains allow VCO to be used for various purposes, especially nutrition.

A recent study reported that lipid may inducing leakage, moreover at the higher concentrations effect on complete disintegration of the envelope and the viral particles (Thormar *et al.*, 1987). Medium chain fatty acids reportedly can be used as prevention compound against porcine epidemic diarrhea virus (PEDV) (Lerner *et al.*, 2020). Moreover, lipid-based therapies for SARS-CoV-2 can be limiting the access of host lipids to the virus, either by using lipid inhibitors or supplementation with exogenous lipids. It might significantly limit the SARS-CoV-2 infection and/or severity (Alketbi *et al.*, 2021a).

Therefore, the objective of this research was to explore whether VCO components are a good candidate to be an inhibitor of SARS-CoV-2, especially with Medium-chain fatty acids (MCFA) against Nsp3b as one of target for virtual ligand screening of potential drug targets for SARS-CoV-2 (Wu *et al.*, 2020).

MATERIALS AND METHODS

Protein preparation

The Nsp3b as one of target for virtual ligand screening of potential drug targets of SARS-CoV-2 (Wu *et al.*, 2020). The existing structure of the (RSCB ID: 7QG7) was retrieved from the RCSB website https://www.rcsb.org/structure/7QG7. The protein was cleaned using YASARA-view, Yet Another Scientific Artificial Reality Application, to remove its 1,2-ethanediol and natural ligands.

Ligand preparation

The sdf files of 5 ligands (Table 1) were collected from PubChem. SwissADME web server (http://www.swissadme.ch/) was used to predict several molecular properties of each ligand, including LogP (Daina *et al.,* 2014), and numbers of hydrogen bond donors as well as acceptors. Lipinski's rule of five was used to screen the ligands. Ligands that violated more than one rule were exlcuded from this study. The ligands were optimized using marvin space software (https://docs.chemaxon.com/display/docs/marvinspace.md) including major microspecies protonation and molecular conforming utilizing.

Quantitative structure-activity relationship (QSAR) analysis

QSAR analysis was conducted to predict the bioactivity of the ligands using Way2drug/PASS server http://www.way2drug.com/PASSOnline/ (Filimonov *et al.*, 2014). Bioactivity scores were collected and proceed to draw heatmap analysis for visualizing bioactivity of each ligand. Several activities related to antiviral activity or antiviral against specific positive single-stranded RNA virus families or viral entry inhibitors were collected to understand bioactivity of ligand.

Binding site for docking

The pdb file of Nsp3b (RSCB ID: 7QG7) had a ligand remdesivir nucleoside GS-441524 (PubChem ID: 44468216) anchored to protein. The pocket (active site) used to bind remdesivir is then used as the binding site for our ligand. Ligand binding site definition is obtained using PLANTS 1.1 software (Korb *et al.,* 2009). This software is also used for molecular docking for our ligands.

Molecular docking

The molecular docking software validation was done with re-docking of natural ligands remdesivir nucleoside GS-441524 (PubChem ID: 44468216) to protein using PLANTS 1.1 software (Korb *et al.,* 2009). The root-mean-square deviation (RMSD) of natural position and position after re-docking is then calculated using YASARA. Docking software which resulting RMSD <2.0Å after ligand re-docking is categorized as valid software. Molecular docking for all ligands were continued after assuring the software validation. Docking score were collected from PLANTS 1.1 software while binding affinity is calculated using KDEEP: Protein–Ligand Absolute Binding Affinity Prediction via 3D-

Convolutional Neural Networks (Jiménez *et al.*, 2018).

Absorption, distribution, metabolism, excretion (ADME), and toxicity prediction

The ADME and toxicity of the ligands were also analysed. The SMILES of the compounds were proceeded to the ADMETlab 2.0 webserver (https://admetmesh.scbdd.com/service/evaluation/cal) (Xiong *et al.*, 2021) and Toxtree (Patlewicz *et al.*, 2008). Several parameters were generated from the server to be analyzed, such as blood-brain barrier, HIA, LC50, AMES toxicity, skin sensitization, carcinogenicity, and eye irritation.

Molecular dynamics simulation

Molecular dynamics (MD) simulation predicts the movement of every atom in the protein to capture changes in protein conformation, ligand binding, and folding of a protein was performed using GROMACS (Abraham *et al.*, 2015).

RESULTS

Lipinski's rule states (Lipinski *et al.*, 2001) that in general, an orally active drug is not had more than one violation of the following criteria:

- No more than 5 hydrogen bond donors (the total number of nitrogens–hydrogen and oxygen–hydrogen bonds)
- No more than 10 hydrogen bond acceptors (all nitrogen or oxygen atoms)
- A molecular mass less than 500 daltons
- An octanol-water partition coefficient [10] (log P) that does not exceed 5

We found myristate and laurate have no violation regarding Lipinski's rule, while palmitate, oleate, and stearate have one violation. The violation of from palmitate, oleate, and stearate were in Log P score. Three compounds including: palmitate, oleate, and stearate were higher than 5 (Table 1).

We found that all compounds have activity as antitoxic with score >0.65 (Figure 1). Several publications were ever mentioned long chain fatty acid. Lipid has been mentioned as therapies against Sars-CoV-2 (Alketbi *et al.*, 2021b).

We were validating our docking method with re-docking ligand to protein. Programs which are able to return poses below Root Mean Square Deviation (RMSD) value from the known conformation (usually 1.5 or 2 Å depending on ligand size) are considered to have performed successfully and showing valid software methods for docking ligands to protein (Hevener *et al.*, 2009). Our redocking score was 0.1843 Å (Figure 2).

We found that binding affinity of ligand control that naturally docked in protein is -5.71. Two of our ligands; stearate and palmitate had better lower binding affinity than ligand control. Both ligands have binding affinity -6.45 and -6.23 respectively. While binding affinity three others ligand: oleate, myristate, and laurate respectively -5.53, -5.43, and -4.60 (Table 2). Our control ligand docking score was also showing difference docking score between ligands. Our control ligand docking score is -82.74. While score for three ligands; palmitate, stearate, myristate, laurate, and oleate were -83.06, -88.10, -78.25, -71.24, -89.08 (Table 2).

We found both palmitate and stearate docked to Nsp3b protein due several residues of amino acid. Four residues were developing four hydrogen bonds between stearate and protein. Amino acid residues which used in hydrogen bonds developing were Val(49), Ala(50), Ala(38), and Gly(47). While palmitate were anchoring two hydrogen bonds from two residues Val(49), and Val(123). Ligand control has more hydrogen bonds than palmitate and stearate. There were five hydrogen bonds between ligand and protein: Ile(23), Phe(156), Asp(157), Leu(126), Asp(22) . However, stearate was shown develop more non-hydrogen bond interaction, such as Alkyl and Pi-Alkyl interaction (Figure 3).

Table 1. Molecular properties of VCO compounds with Lipinski's rule.

No.	PubChem ID	Compound	SMILES	Lipinski's rules				Violation
				Hydrogen bond donors	Hydrogen bond acceptors	Molecular mass (Dalton)	LogP (consensus)	
1	985	Palmitate	CCCCCCCCCCCCCCCC(=O)O	1	2	256.42	5.20	1 violation
2	5281	Stearate acid	CCCCCCCCCCCCCCCCCC(=O)O	1	2	284.48	5.93	1 violation
3	11005	Myristate acid	CCCCCCCCCCCCCC(=O)O	1	2	228.37	4.45	No violation
4	3893	Laurate acid	CCCCCCCCCCCC(=O)O	1	2	200.32	3.51	No violation
5	445639	Oleate acid	CCCCCCCC/C=C\CCCCCCCC(=O)O	1	2	282.46	5.65	1 violation

Table 2. Molecular docking and binding affinity score of ligands

No	Molecule	Docking Score	Binding Affinity ΔG (kcal/mol)
1	Control ligand/remdesivir (PubChem ID: 44468216) (PDB: 7QG7)	-82.74	-5.71
2	Oleate acid (PubChem ID: 445639)	-89.08	−5.53
3	Palmitate (PubChem ID: 985)	-83.06	−6.23
4	Stearate acid (PubChem ID: 5281)	-88.10	−6.45
5	Myristate acid (PubChem ID: 11005)	-78.25	−5.43
6	Laurate acid (PubChem ID: 3893)	-71.24	−4.60

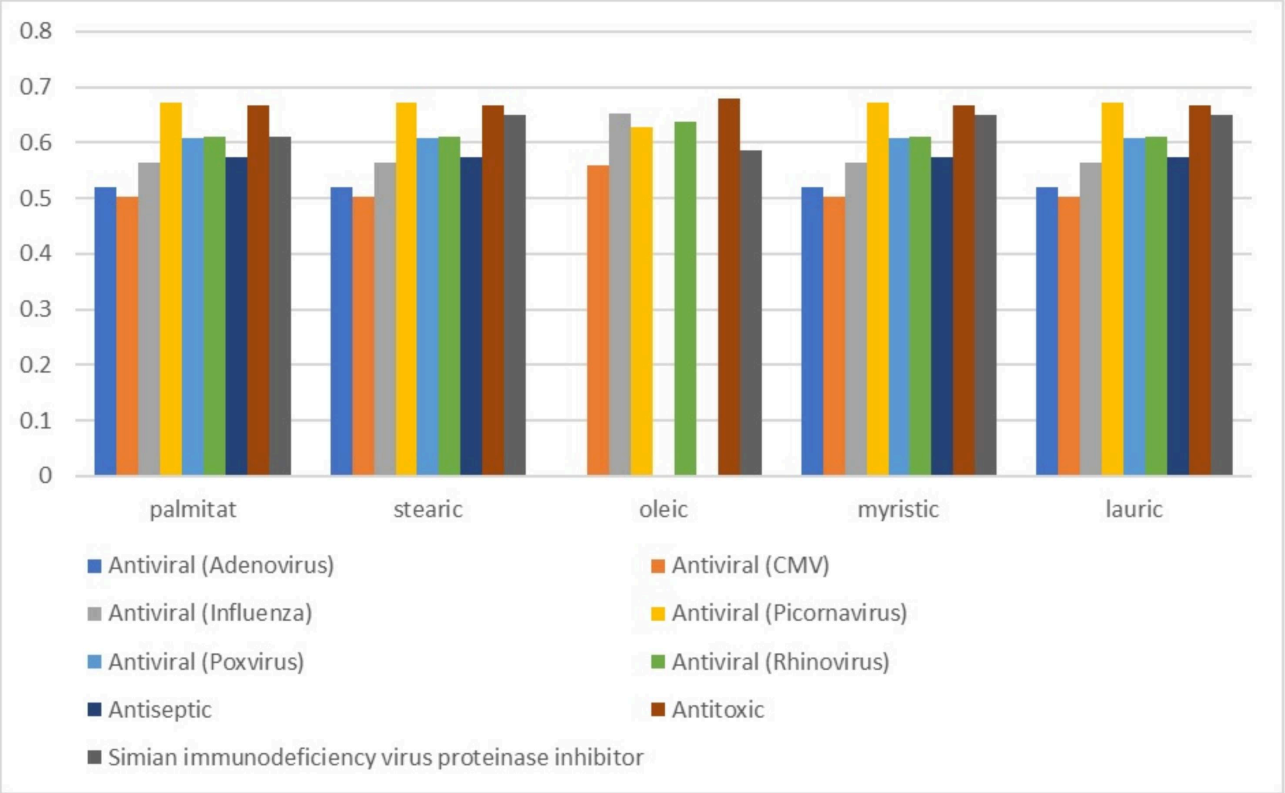

Figure 1. Quantitative structure-activity relationship (QSAR) analysis using Way2drug/PASS server.

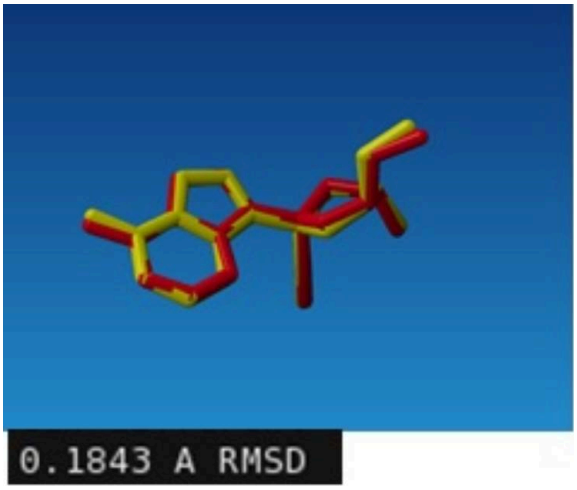

Figure 2. Re-docking original ligand (PubChem ID: 44468216) for software validation.

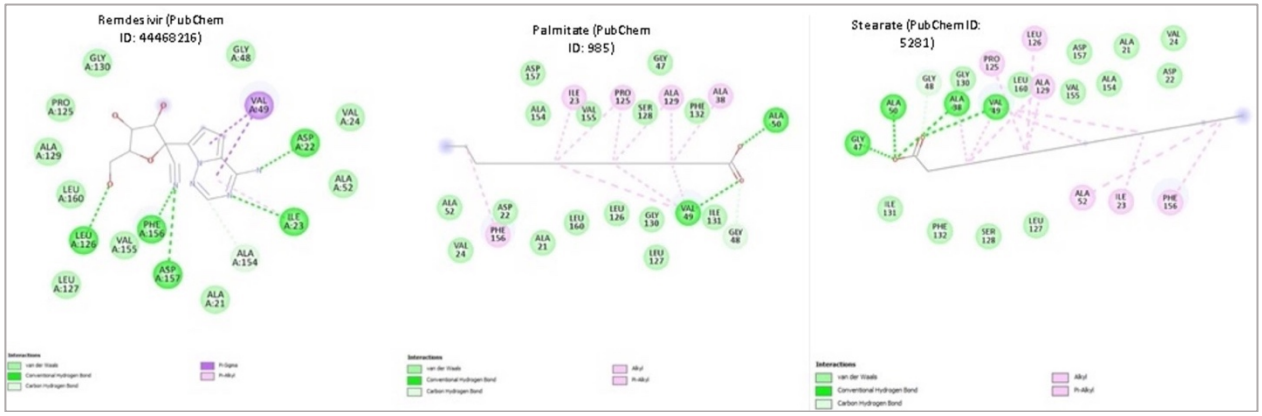

Figure 3. The 2D Structure interaction between ligand.

Table 3. Hydrogen bond interaction between certain ligands.

No	Ligand	Hydrogen donor	Hydrogen acceptor	Distance (Å)
1	Natural (Pubchem ID: 44468216)/U08201	ILE(23)	U08201: N4	2.95
		PHE(156)	U08201: N3	3.28
		ASP(157)	U08201: N3	3.18
		U08201: O4	LEU(126)	2.48
		U08201: N5	ASP(22)	2.70
2	Palmitate (PubChem ID: 985)/PAL	VAL(49)	PAL: O2	2.60
		ALA(50)	PAL: O1	2.74
3	Stearate acid (PubChem ID: 5281)/STE	VAL(49)	STE:O2	2.09
		ALA(50)	STE:O1	2.08
		STE:O1	ALA(38)	3.22
		STE:O1	GLY(47)	3.29

We also calculated distance between ligands and protein to gain more information about the interaction. We found that average interaction distance between stearate and protein was closer in distance than ligand control to protein. However total hydrogen bonds between ligand control and protein were more than protein to palmitate or protein to stearate. Interaction image (Figure 4) were showing the distance difference between two ligands also ligand control with protein. However, this position is only showing one condition interaction clip between the control ligand and Nsp3b protein as well as stearate and Nsp3b protein. Molecular dynamic simulation is needed to understand more about molecule interaction movement during simulation (Singh *et al.,* 2022)

ADMET describe of chemical absorption, distribution, metabolism, excretion, and toxicity. These factors play important roles in drug discovery and drug development. A drug can be determined as potentials compound by having the proper ADMET values when it is given at a therapeutic dose and resulting a high efficacy activity to the target (Table 4).

During molecular dynamic simulation, the stability of the protein back bone (bb), protein–ligand complex (com) and the ligand were scrupulously studied. The RMSD of the protein backbone has rendered below 0.175 nm and the RMSD of the ligand has rendered below 0.45 nm. The RMSD backbone which docked palmitate was noted to be deflected at around 7.5 ns, while backbone of protein which docked to stearate shown flat since 3 ns (Figure 4A). The RMSD of the complex protein to ligand: palmitate and stearate were shown relatively difference. The RMSD of complex protein to stearate was stable below 1 nm, while protein with palmitate complex was shown two times fluctuations. There were between 7 to 8 ns and 11 to 13 ns and dropped thereafter, remaining relatively stable. This deviation is presumed to be due to the palmitate ligand that has been adjusting at the binding pocket of the protein (Figure 4B). The RMSD of the ligand relatively stable for two ligands: palmitate and stearate. The highest RMSD score for two ligands was 0.45 nm. Both of ligands were shown stable around 0.35 nm (Figure 4C).

The radius of gyration (Rg) is describing the compactness of the protein. It was measured for the protein backbone. The Rg of both the systems was found to be ranging between 1.46 nm and 1.51 nm during the simulation (Figure 4D). The average Rg for both ligand: palmitate and stearate was computed as 1.48 nm.

The fluctuations of residue atoms protein backbone were measured by RMSF. The backbone residues have calculated low fluctuations. The RMSF of the protein residues was projected to be below 0.2 nm (Figure 5A). It was implying that the protein backbone was constantly stable during simulation. This result was supporting the backbone RMSD and the gyration results. Both backbone palmitate and stearate were shown a minor peak was noticed with residues Gly46, Gly47, Gly48, and Val49 when compared with the other residues reaching to about 0.17 nm. Residue Gly47 exist in proximity of the binding pocket both palmitate

and stearate, while Val 49 was found in stearate binding pocket, which was binding to protein during molecular (Figure 3).

During molecular simulations, the formation of the hydrogen bonds was observed. The hydrogen bonds were formed throughout the simulations. It is suggesting that the ligands remain in the binding pocket during the entire simulation. The average hydrogen bond number for palmitate was about 0.2. The stearate, on the other hand, has generated more hydrogen bonds throughout the simulation run. The average hydrogen bonds were revealed 1.2. It is noted that the hydrogen bonds were recorded to be higher during the last 10 ns with an average of 1.5. It is implying that the ligand has firmly occupied the active site (Figure 6B).

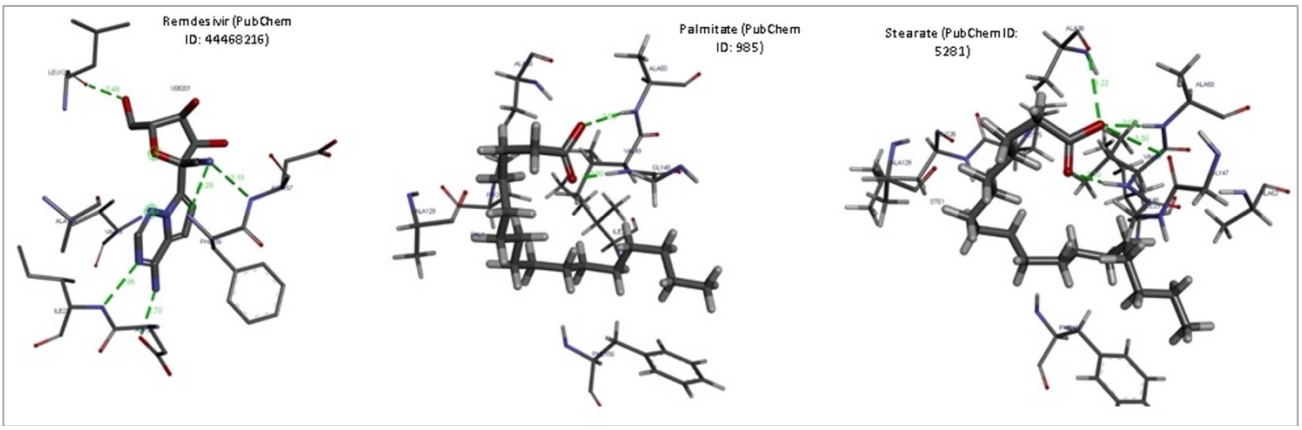

Figure 4. The 3D Structure interaction between ligand and protein.

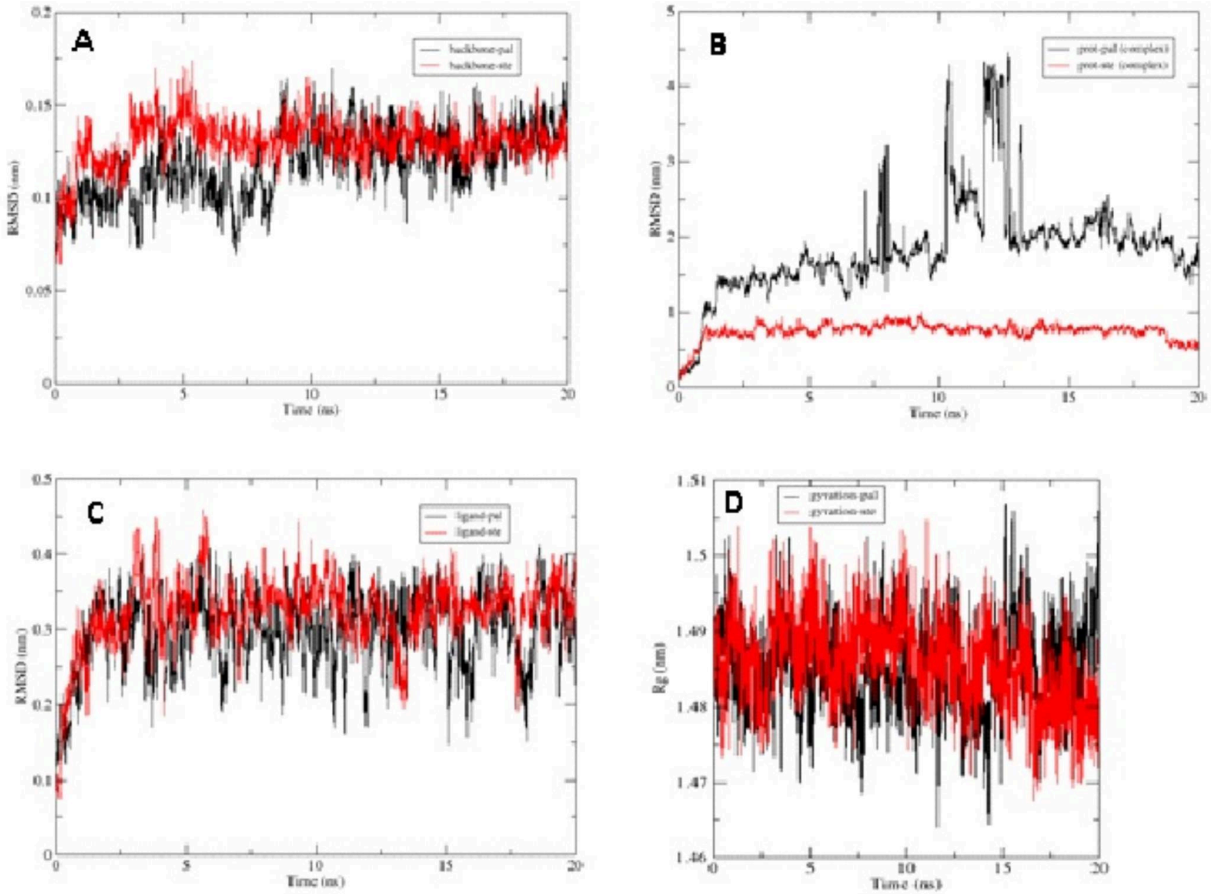

Figure 5. MD simulation inferred stability results. (A) Root mean square deviation of the protein backbone (B) RMSD of the protein-ligand complex. (C) RMSD of the ligand. (D) Compactness analysis according to Rg (radius of gyration).

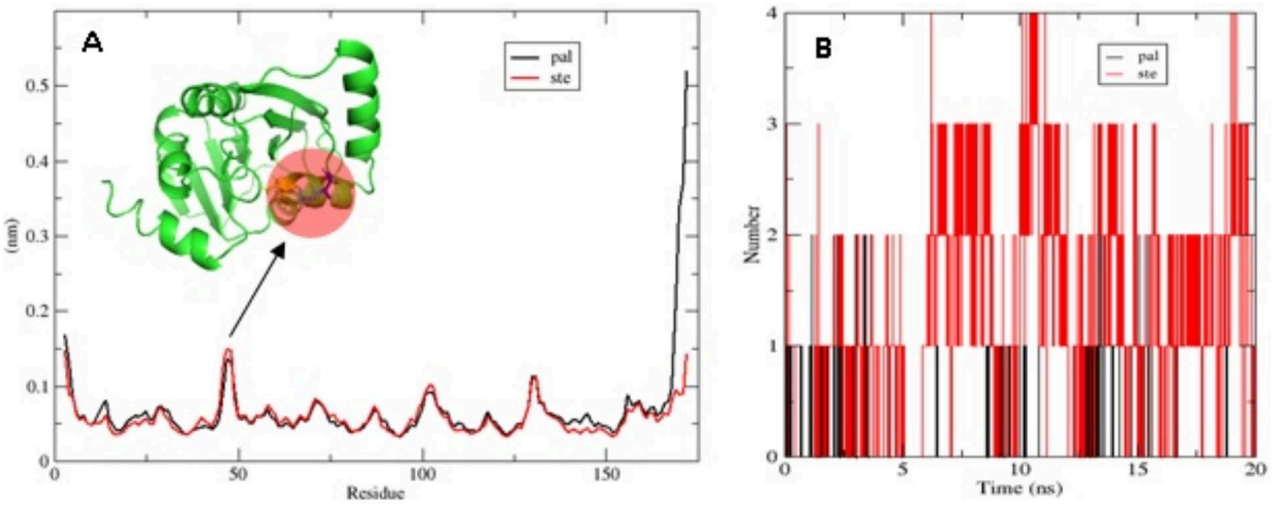

Figure 6. (A) Root mean square fluctuations. The binding pocket is represented in red sphere (B) Hydrogen bond number during the simulation.

DISCUSSION

We found that all compounds have biological activity against picornavirus with scores >0.6. Prediction score rate from 0 to 1 (Filimonov *et al.*, 2014). All compounds were predicted as antiviral against rhinovirus with scores >0.6. Laurate, palmitate, stearate, and myristate have biological activity against for adenovirus, influenza, poxvirus, CMV and immunodeficiency virus. However, the only compound which have not biological compound against adenovirus and poxvirus is oleate. Oleate is also the only compound that has not biological activity as antitoxic antiseptic.

One of the most frequently mentioned aspects of ADME is human intestinal absorption (HIA). HIA is commonly used to study the use of pharmaceuticals in the human body regarding to statistical models. HIA is also a crucial stage in the delivery of drugs to their right destination (Yan *et al.,* 2008). This study shows that both stearate and palmitate had good absorption since ≥ 30% were absorbed (Table 4) (Yan *et al.,* 2008). Better score of absorption lead to better bioavailability and may suggest that the compounds are complete the oral administration.

The ADMET results also show negative for BBB barrier. It is indicating that both palmitate and stearate will not cause toxicity to the brain as they cannot pass through the BBB barrier. It can prevent the entry of drugs into the brain from the blood (Table 4) (Pardridge, 2012). Palmitate and stearate were also shown to have negative carcinogenicity, meaning compounds unlikely to cause cancer. Ames toxicity analysis for palmitate and stearate was also shown negative for both of compounds. Ames toxicity is used to assess the potential carcinogenic effect of chemicals (Jain *et al.*, 2018). The compounds also shown low (class I) toxicity in creamer rules decision tree and revised creamer rules decision tree (Lapenna & Worth, 2011). The decision tree categorizing compund due to basis of chemical structure and reactivity of the chemicals. Classification is built into three classes indicating a high (Class III), medium (Class II) or low (Class I) level of concern where the lower of class the more harmless of the compound (Table 4).

In addition, palmitate, and stearate LC50FM showed values of 5.246 and 5.011, respectively, which is a relatively low number which indicates the effective killing of 50% of fathead minnow. On the other hand, the LC50DM score showed values of 4.357 for palmitate and 4.605 for stearate. LC50DM is a score which show the value effective to kill 50% of *Daphnia magna* (Table 4). The last analysis was skin sensitization and eye irritation. This test is showing both of palmitate and stearate may have possible effect on skin and eye irritation, but it is often not discussed for oral medicine (Table 4).

Table 4. Absorption, distribution, metabolism, excretion (ADME), and toxicity prediction.

No	Compound	ADMETlab 2.0 webserver								Toxtree	
		Ames toxicity	**Skin sensitization**	**Carcinogenecity**	**Eye irritation**	**LC50FM**	**LC50DM**	**HIA**	**BBB penetration**	**Creamer rules decision tree**	**Revised creamer decision tree**
1	Palmitate	---	++	---	+++	5.246	4.357	---	---	Low (class I)	Low (class I)
2	Stearate acid	---	+++	---	+++	5.011	4.605	---	---	Low (class I)	Low (class I)
3	Myristate acid	---	++	---	+++	5.091	4.127	---	--	Low (class I)	Low (class I)
4	Laurate acid	---	+	---	+++	4.462	3.939	---	-	Low (class I)	Low (class I)
5	Oleate acid	---	+++	---	+++	4.794	4.470	---	--	Low (class I)	Low (class I)

CONCLUSION

This study showed stearate and palmitate as the most potential candidate for Nsp3 inhibitors. Palmitate and Stearate were readily absorbed by the intestines and predicted cannot penetrate the blood-brain barrier. Palmitate and stearate passed all the toxicology criteria. Even though the molecular dynamic results showed unstable RMSD for palmitate complex, the QSAR analysis described that compound was likely to elicit antiviral activity and bind to the target protein based on the molecular docking binding affinity score. However, further investigations through wet-lab experiments such as in vivo are necessary needed.

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CONFLICT OF INTEREST

The authors have declared that no conflict of interest exists.

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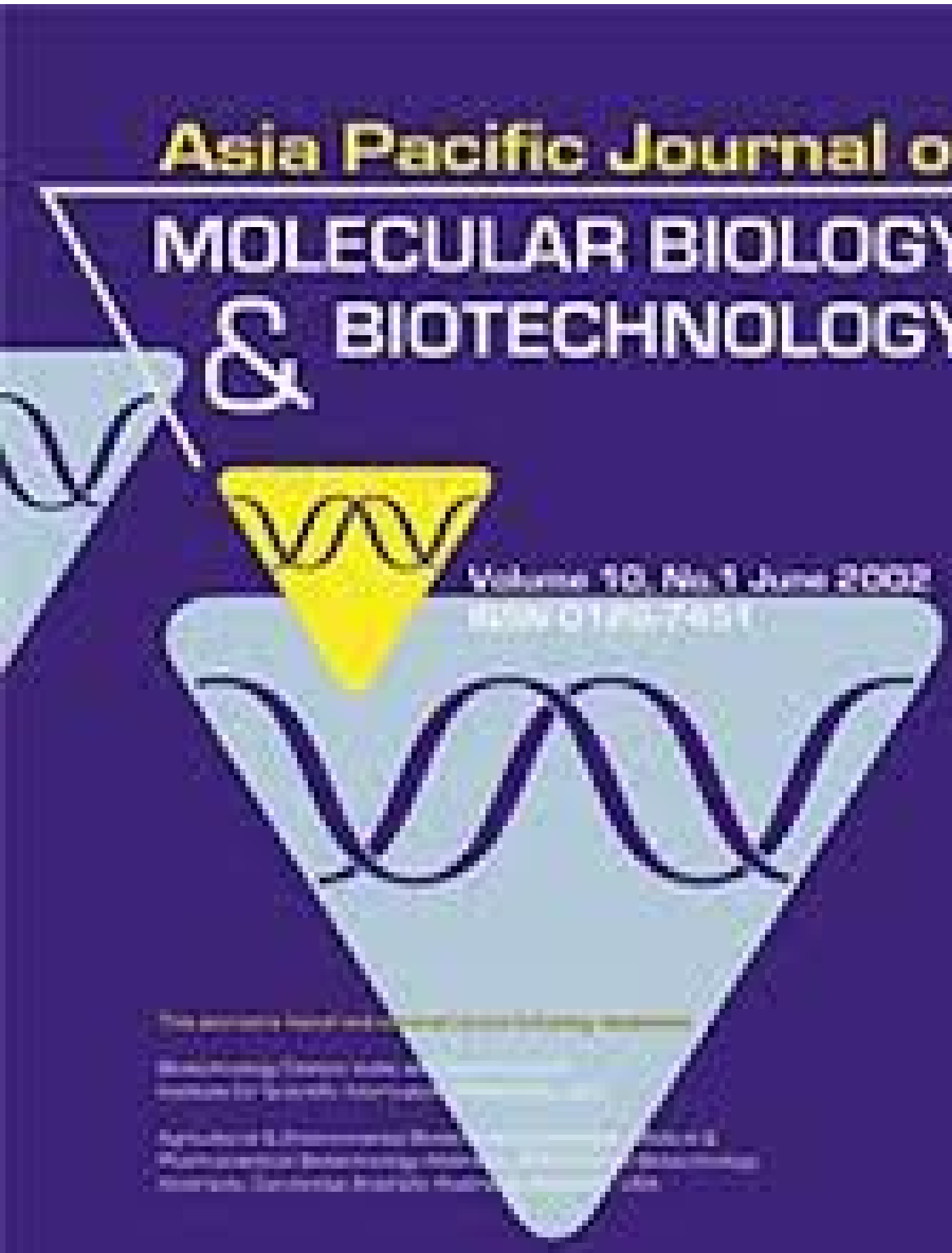

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Michelle Teo Yee Mun is currently a lecturer in the Faculty of Applied Sciences, UCSI University. Previously, she obtained her BSc and MSc from the University of the West of England in Bristol, United Kingdom, followed by a PhD from UCSI University. Her research interests are molecular oncology and cancer immunology, with specialization in immunotherapies such as immunotoxins and antibody engineering. Michelle has been actively participating in various international and national conferences and symposiums and received several awards, including the Best Abstract Award during the Hong Kong Croucher Summer Course in 2018.

Editorial Board Members

Khatijah Yusoff, FASc, FIAS, FTWAS; PhD; DSc (hon causa) (La Trobe)

Khatijah has over 32 years' experience investigating the molecular virology of Newcastle disease virus. Her current research interest is in in the development of NDV as a therapeutic oncoviral vaccine through the use of reverse genetics. She is also involved in the molecular biology of other organisms, in particular on the use of nanobiotechnology, surface display technology and phage therapy. She is currently in the Vice-President of World Academy of Sciences (TWAS); and sits in the Councils for the Academy of Sciences Malaysia, the Islamic World Academy of Sciences, TWAS, and the Council of Science Advisers (CSA) of the International Centre for Genetic Engineering and Biotechnology (ICGEB).

Lau Yee Ling, PhD

Professor Dr Lau Yee Ling is at present the Head of the Department of Parasitology, Faculty of Medicine at University of Malaya (UM). She started her academic career as a lecturer at Monash University Sunway Campus while waiting for her PhD viva in 2008. During her time as a lecturer in Monash University, she was awarded two Monash University Research Grants in which enabled her to continue her research in the field of molecular parasitology. She then returned to her alma mater, University of Malaya, as a Senior Lecturer in 2009. She was granted tenure in 2010 and promoted to Associate Professor in 2013, and Professor in 2019. Professor Lau has been awarded University of Malaya Excellent Service Award three times in 2011, 2013 and 2015. She was awarded MSPTM Nadchadtram Medal in 2014. She has also been awarded a few times for her innovation in research including the Grand Prize in National Exclusive Innovation Challenge Award 2018.

Suet Lin Chia, PhD

Suet Lin obtained his PhD in the field of Medical Biotechnology from Universiti Putra Malaysia in year 2012 under the supervision of Prof. Dr. Khatijah Yusoff. He underwent a two-year post-doctoral training in the Seymour's lab in the University of Oxford. His field of interest is to develop a potent oncolytic Newcastle disease virus for the treatment of cancer. Currently, his research involves genetic modification of NDV genome to reduce the virus pathogenicity towards birds. In addition, he is also working on cloning immunostimulatory genes into the virus backbone to enhance the oncolytic properties of the virus.

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JayaprakashArulraj, Ashokraj Kattur Venkatachalam, Revathy Soundararajan, Rajesh Embranahalli Mani

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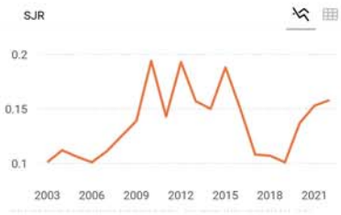

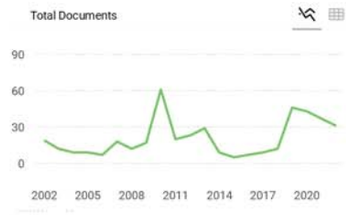

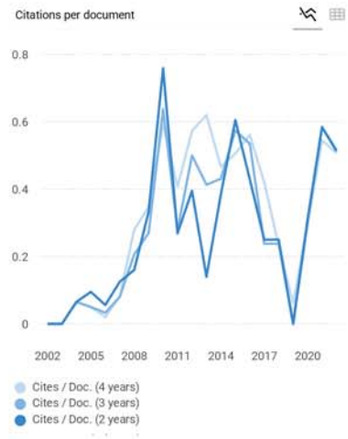

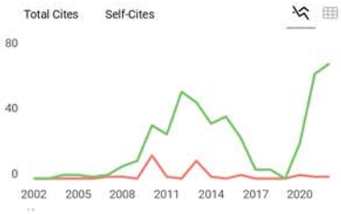

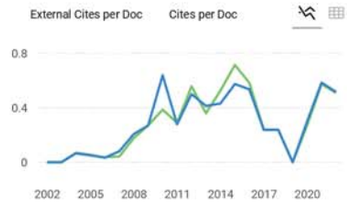

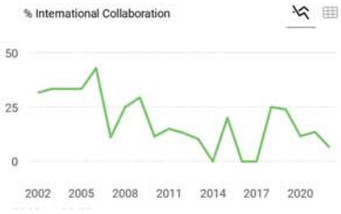

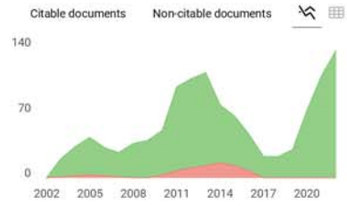

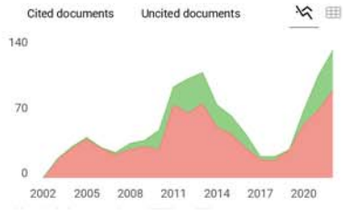

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D **Dr. Suet-Lin Chia** 5 years ago

The official website for the journal is http://www.msmbb.my/index.php/publication. For any inquiries on the journal, please contact us at apjmbb@upm.edu.my.

Thank you.

Suet-Lin Chia, PhD
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Source details

Asia-Pacific Journal of Molecular Biology and Biotechnology

Scopus coverage years: from 2002 to Present
Publisher: Universiti Putra Malaysia
ISSN: 0128-7451
Subject area: Biochemistry, Genetics and Molecular Biology: Biotechnology
Biochemistry, Genetics and Molecular Biology: Molecular Biology
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