JARES, Vol.9 No.1 Maret 2024
p-ISSN: 2502-826X / e-ISSN: 2503-1163
DOI: https://doi.org/10.35457/jares.v9i1.1363

IN SILICO STUDY : HERMETIA ILLUCENS BASED ACUTE RESPIRATORY INFECTION TREATMENT

Diterima:
5 Februari 2024
Revisi:
10 Maret 2024
Terbit:
11 Maret 2024

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Abstract— *The novel coronavirus is now referred to as severe and critical acute respiratory syndrome coronavirus-2 (SARS-CoV-2). There have been few studies about SARS-CoV-2 co-infection may significantly inhibit the immune system of host, increase antibacterial therapy intolerance, and be harmful to the prognosis of the disease. The highest co infection comes from Streptococcus pneumoniae. Bacterial co-infection in the setting of viral pneumonia is known as major cause of mortality. Therefore, therapeutics such as antibiotics are needed to be able to kill and inhibit these bacteria. In this connection, the inaccurate use of antibiotics causes multi-drug resistant and worsens their immature immune systems. Hermetia illucens contains AMPs and various amino acids that synergistically have the potential to overcome this problem. Research on the use of crude maggot extract as a candidate for acute respiratory infection treatment products is still not available and has never been reported. This study aims to conduct in silico computerized tests related to the potential of maggot extract as antibacterial and anti-inflammatory properties, analyze its interaction with target proteins, bioavailability and ligand toxicity in maggot extract, and docking analysis of ligand-receptor. The results showed that the maggot extract had activity as peptidoglycan glycosyltransferase inhibitor, antibacterial, and anti-inflammatory. A binding affinity of the maggot AMPs ligands (defensin, diptericin, and attacin) to MurC receptor protein Streptococcus pneumoniae is also found. The antibacterial and anti-inflammatory abilities of bioactive maggots have the potential for used as a candidate treatment products for SARS-CoV-2 co-infection with Streptococcus pneumoniae as a biomedical innovation.*

Keywords— *Acute respiratory infection, Hermetia illucens, in silico, Streptococcus pneumoniae*

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p-ISSN: 2502-826X / e-ISSN: 2503-1163
DOI: https://doi.org/10.35457/jares.v9i1.1363

I. INTRODUCTION

The prevalence of acute respiratory infection (ARI) cases continue to increase and become a global health threat. Respiratory illness caused by a novel coronavirus was first noted in December of 2019 in Wuhan, Hubei Province, China [1]. The novel coronavirus is now referred to as severe and critical acute respiratory syndrome coronavirus-2 (SARS-CoV-2) was transmitted through respiratory tract and could induce pneumonia. By 6 April, WHO has reportedof1, 210, 956 laboratory-confirmed cases of SARS-CoV-2 infection and 67,594 deaths worldwide [2]. The current outbreaks of coronavirus infection remind us that CoVs are still a severe and critical threats to global public health. This condition certainly is the antithesis of the 3rd goal in SDGs, good health and well-being. There have been few studies about SARS-CoV-2 where co- infection may significantly inhibit the immune system of host, increase antibacterial therapy intolerance, and be harmful to the prognosis of the disease. The highest co infection comes from *Streptococcus pneumoniae*. Bacterial co-infection in the setting of viral pneumonia is known as major cause of mortality. SARS cov-2 can also synergize with *Streptococcus pneumoniae*, so that it can suppress the host immune system through a series of biological processes [3].

Therefore, therapeutics such as antibiotics are needed to be able to kill and inhibit these bacteria. In this connection, the inaccurate use of antibiotics causes multi-drug resistant and worsens their immature immune systems. In this connection, the inaccurate use of antibiotics, especially in children and toddlers, causes multi-drug resistant and worsens their immature immune systems [4]. Therefore, we need innovation and efforts in the biomedical sector to tackle the problem of acute respiratory infection in Indonesia. The form of biomedical innovation that will be invented is expected to have an influence in killing and inhibiting *S. pneumoniae* (highest co infection SARS Cov-2) optimally. The alternative chosen in this study is use a maggot or black solder fly larvae (*Hermetia illucens*) as a new breakthrough in the form of a candidate for acute respiratory infection treatment products.

The reason for choosing maggot (*Hermetia illucens*) as this research material is because the material is abundant and there a place for livestock, also based on the research from which states that maggots contain many and various peptides which are offered as antimicrobials as attacin, defensin, diptericin, cecropin, drosocin, drosomycin, and metchnikowin [5]. Peptides are a group of compounds that can be used as an alternative to conventional antibiotics to eradicate various pathogenic microbes. Antimicrobial peptides (AMPs) are a group of compounds that have the potential to be developed in overcoming the problem of bacterial resistance to conventional antibiotics [6]. These variations of amino acid contents in a maggot

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are also thought to be able to boost the formation of immune cells in the body, making it suitable of use for infants and toddlers with immature immune systems [7]. Besides that, other components of maggot such as α- tocopherol, α-tocotrienol, β-sitosterol, chitin, adipic acid, tannins, β-tocopherol, γ- tocopherol, β-tocotrienol, and γ-tocotrienol which have the potensial as an antibacterial and anti-inflammatory [8]. Research on the use of maggot (*Hermetia illucens*) extract as a candidate for acute respiratory infection treatment product is still not available and has never been reported to exist. Therefore, it is necessary to conduct research to *in silico* analyze the potential of maggot extract as a new breakthrough in the biomedical world in the form of a candidate for the acute respiratory infection treatment product.

This study aimed to testing the ability of the bioactive compound maggot *Hermetia illucens* using *in silico* method against *Streptococus pneumonia* which is the main co-infection of COVID-19 in acute respiratory infections.

II. METHODS

1. Pass Server Computerized Test (Modified Method of Filimonov et al., (2014))

Pass server is a *in silico* software that is used to analyze the antibacterial potential of 10 maggot compounds (except AMPs) obtained from literature studies. The SMILE (Simplified Molecular-Input Line-Entry System) structures of the 10 compounds were searched in the Pubchem database (https://pubchem.ncbi.nlm.nih.gov/), then inputted into the WAY2DRUG PASS prediction (http://pharmaexpert.ru/PASSonline/index.php) via institutional email account.

2. STITCH DB Pathway Prediction Computerized Test (Modified Method of Szklarcyk et al., (2016))

STITCH DB version 5.0. is a *in silico* software that is used to analyze the interaction between 10 maggot compounds and *S. pneumoniae* D39 organism proteins as targets. The result will provide pathway predictions that are visualized with a variety of colors with different meanings. Predictive scores are obtained from the STITCH algorithm and are synthesized from published research results. The description of the role of protein function was obtained from the results of the STITCH and UNIPROT (https://www.uniprot.org/) analysis.

3. Docking Analysis of Ligand-Receptor (Rakhmetov et al., 2016)

The docking method is a molecular modeling simulation to efficiently predict the interactions that occur between the receptors and ligands bound to form a complex. Docking is done by entering ligand and receptor proteins in the (.PDB) format into the Cluspro 2.0

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software via an institutional email account. ClusPro 2.0 will simultaneously generate four types of models using a scoring algorithm known as balanced, electrostatic-favored, hydrophobic-favored, and van der Waals + electrostatic. Pick the first ten relatively low energy docking structures rated by the server. Visual representation of docking results and assessment of complex interactions was performed using Pymol. The docking results were then analyzed the interacting protein residues through the KFC 2 Server (Protein Interface Hotspot Prediction) (http://kfc.mitchell-lab.org) by entering the protein chain list and docking complex results in the (.PDB) format.

4. Ligands Bioavailability and Toxicity Prediction Computerized Test

The ligands from the virtual screen were then analyzed for their solubility and permeability based on Lipinski's rules by accessing the http://swissadme.ch/index.php page. Ligands that passed the Lipinski regulation are then measured for their toxicity by accessing the http://lmmd.ecust.edu.cn/admetsar1/predict/ page. The SMILES structure on the predicted ligands need to be uploaded first to that page, then select the prediction icon.

III. RESULTS AND DISCUSSIONS

1. Result of Pass Server Analysis

Based on the prediction of PASS on 10 *H. illucens* maggot active compounds (**Table 1**), the average PA value was obtained (**Fig. 1**), it can be said that the compounds in maggots have an antibacterial role (0.354) by inhibiting the formation of peptidoglycan (0.481). Besides that, maggots also have an anti- inflammatory role (0.699) which is useful in acute respiratory infection treatment. As additional information based on the pass server test, 10 these compounds were also detected as antiviral (0.58560 through inhibition of RNA synthesis (0.298). The ability of maggot compounds to inhibit viral synthesis is quite potential as a candidate for the SARS-CoV-2 virus drug in cases of corona virus disease 19. The two abilities of the bioactive maggot compounds have a synergistic relationship when it comes to current healrh cases, namely COVID-19. This synergistic relationship is shown by its ability to inhibit the growth of *Streptococcus pneumoniae* which is the main co-infection in COVID-19 cases. If the Pa value is more than 0.7, it indicates that the compound is predicted to have a high potential as antibacterial. Meanwhile, if the Pa value is more than 0.3 but less than 0.7, then the compound has potential as an antibacterial but has allow similarity to
compounds that have been proven to be antibacterial [9]. The compounds were then analysed for their target proteins using the STITCH database (http://stitch.embl.de/). The compounds in

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DOI: https://doi.org/10.35457/jares.v9i1.1363

the *H. illucens* maggot are predicted to interact with proteins that play a role in peptidoglycan synthesis. Peptidoglycan was chosen because the PASS analysis showed potential as a peptidoglycan glycosyltransferase inhibitor in *Streptococcus pneumoniae* bacteria.

Table 1. The PASS Server Test Results on 10 Active *H. illucens* Maggot Compounds

Compound	Antibacteria	Anti-inflammatory	Peptidoglican glycosyltransferase inhibitor	Anti-infective	RNA synthesis inhibitor	Antiviral
α-tocopherol	0.214	0.814	0.215	0.277	0,22	0,386
α-tocotrienol	0.335	0.866	-	0.235	0,199	0,661
β-sitosterol	0.283	0.467	0.78	-	0,3	0,547
Chittin	0.585	0.383	0.322	0.726	0,435	0,712
Adipic acid	0.292	0.51	0.843	0.595	0,344	0,67
Tannins	0.586	0.735	0.245	0.918	0,506	0,676
β-tocopherol	0.296	0.756	-	0.422	0,254	0,403
γ-tocopherol	0.211	0.775	-	0.298	0,26	0,46
β-tocotrienol	0.404	0.835	-	0.365	0,229	0,3845
γ-tocotrienol	0.336	0.846	-	0.251	0,235	0,2935

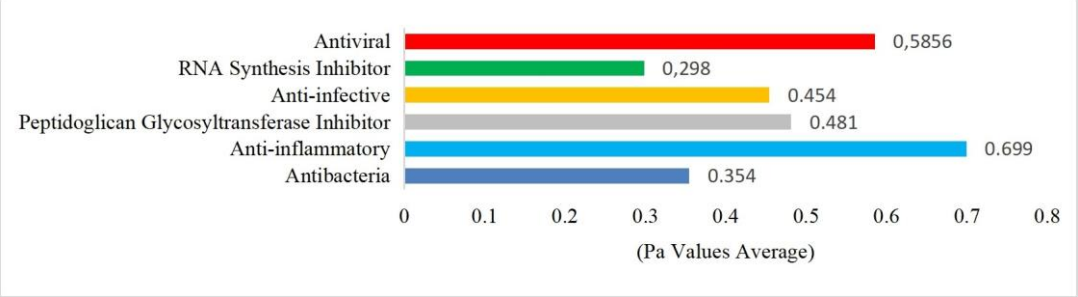

Fig. 1. The Relative Score of *H. illucens* Maggot Pass Server

2. Result of STITCH DB Analysis

The compounds were then analyzed for their target proteins using the STITCH database (http://stitch.embl.de/). The compounds in the *H. illucens* maggots are predicted to interact with proteins that play a role in peptidoglycan synthesis. Peptidoglycan was chosen because the

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PASS analysis showed potential as a Peptidoglycan glycosyltransferase inhibitor in *S. pneumoniae* bacteria. Based on the STITCH DB analysis (**Fig. 2**), compounds in maggot through phosphate can interact with **FabZ** (3-hydroxyacyl-acyl-carrier-protein) which plays a role in unsaturated fatty acid biosynthesis. Therefore, maggot compounds also have interactions with proteins that play a role in cell wall formation, cell shape regulation, and peptidoglycan biosynthesis, these proteins are **murC** (UDP-N- acetylmuramate-L-alanine ligase),**murG**(UDP-N-acetylglucosamine-N-acetylmaramyl-(pentapeptide)pyrophosphoryl-undecaprenol N-acetylglucosamine transferase), and **murB** (UDP-N-acetylenolpyruvoylglucosamine reductase). While other compounds are fat groups that function as antioxidants and affect the synthesis of bacterial plasma membranes, such as chitin and sitosterol.

Peptidoglycan is a major component of the cell wall of almost all eubacteria, including *Streptococus pneumonia.* Structurally, peptidoglycan consists of a linear glycan chain with N-acetyl glucosamine and N-acetyl muramic acid units bound by a trans peptide bridge. In connection with that, in the biosynthesis of one of the peptidoglycan glycans, namely N-acetyl muramic acid, is assisted by the mur enzyme group. The mur enzyme group that helps the process of peptidoglycan formation in *Streptococus pneumonia* has been identified through STTICH DB testing, namely murB, murC, and murG. Based on the results obtained, it can be seen that only murC has catalytic properties function . Meanwhile, murB only functions as a binder and the function of murG is still unknown. The function of the catalyst is very important in the synthesis process, if this part is inhibited, it will certainly disturb the stability of the resulting cell wall. This is related to the function of the murC enzyme as a nonribosomal peptide ligase which catalyzes the addition of L-Alanine to the acetyl muramyl-L-alanine nucleotide UDP-N nucleotide precursor UDP-N. If Nut C is inhibited from working, it will cause deformity of the glycan chain [10]. Therefore, murC was chosen as the focal receptor in this study.

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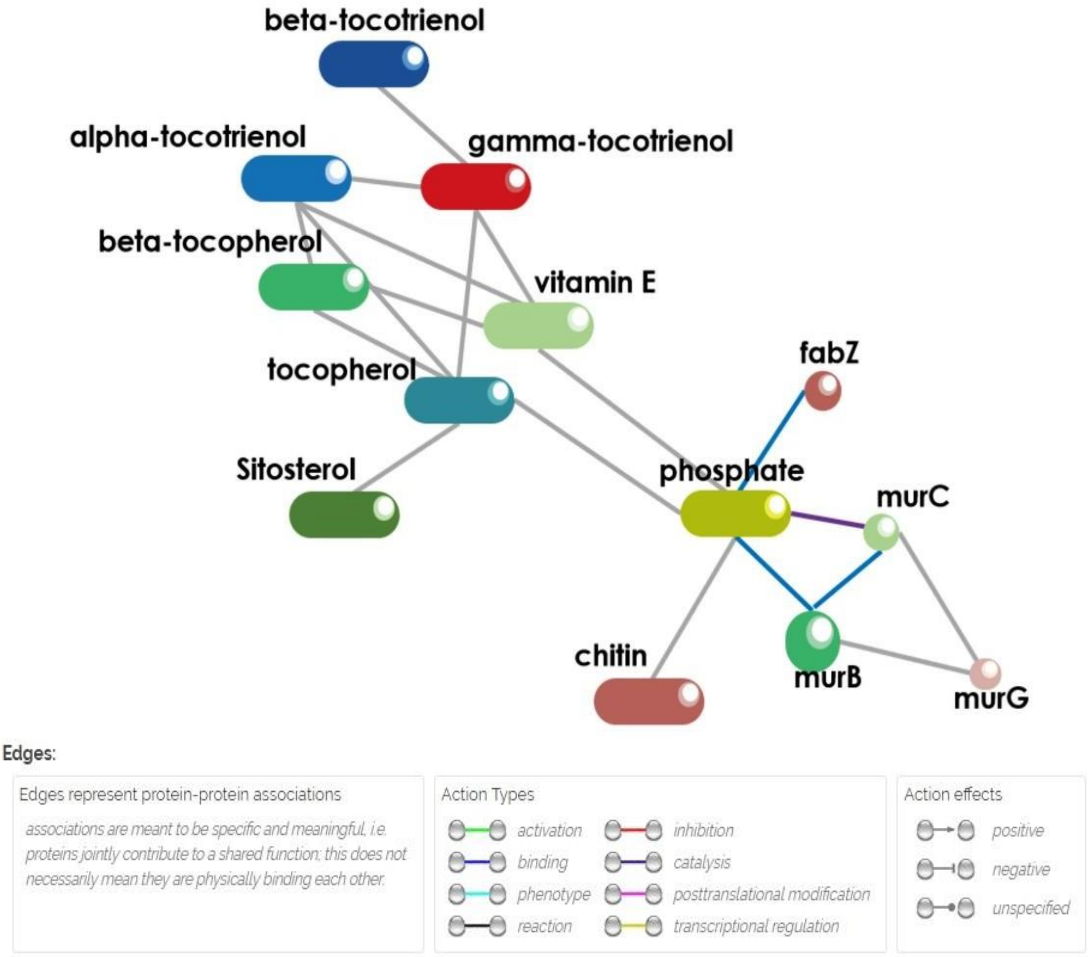

Fig. 2. The Result of STITCH DB Interactions

3. Docking Analysis Result of Ligand-Receptor

Docking was performed using Web Cluspro 2.0 to determine the interaction of the murC protein from *S. pneumoniae* as a receptor and AMPs as protein-ligands. MurC protein was chosen because it plays a role in cell wall formation, cell shape regulation, and peptidoglycan biosynthesis in *S. pneumoniae* bacteria. AMPs from *H. illucens* maggots used were defensin, diptericin, and attacin. Based on research by Chambers *et al.* [11], drosomycin is a protein that has been shown to affect *S. pneumoniae* bacteria, so that this protein is used as a positive control ligand. The results of the docking analysis are presented in various interaction models in **Fig. 3**, to determine which interaction model to use is the lowest energy (**Fig. 4**)

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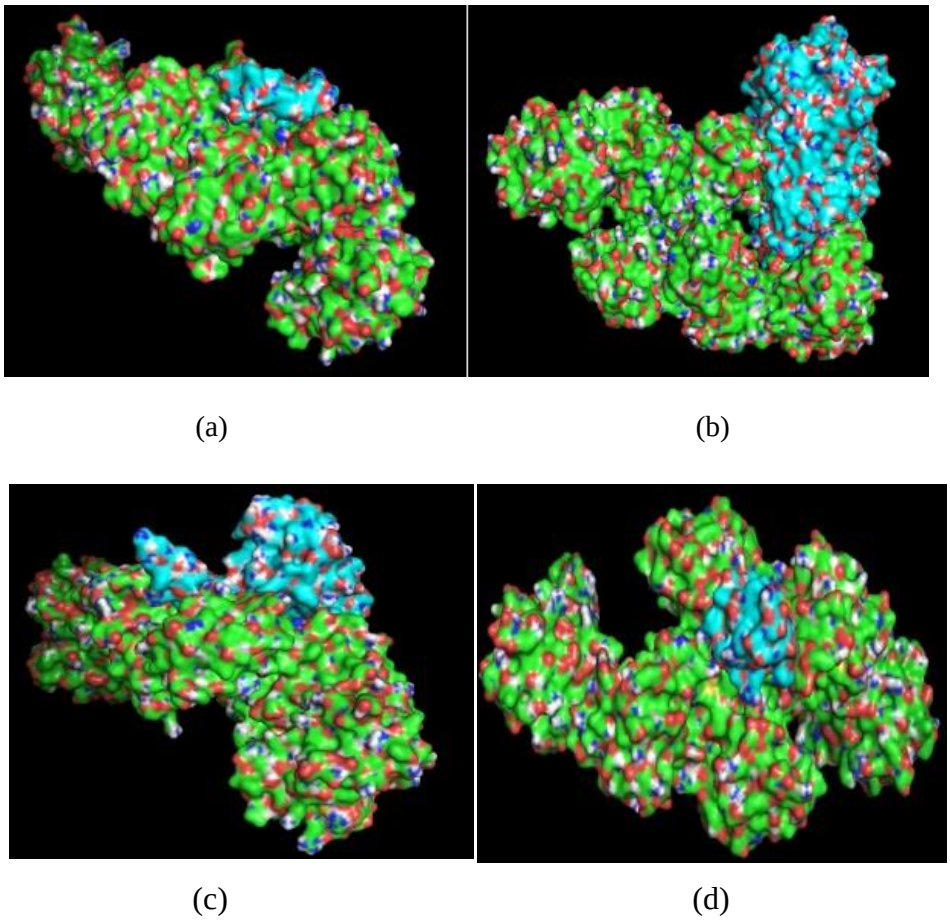

(a) (b)

(c) (d)

Fig. 3. Cluspro 2.0 Result of Ligan-Receptor Interaction Models a.) Defensin- murC b.) Diptericin-murC c.) Attacin-murC d.) Drosomycin (Control Ligand)- murC

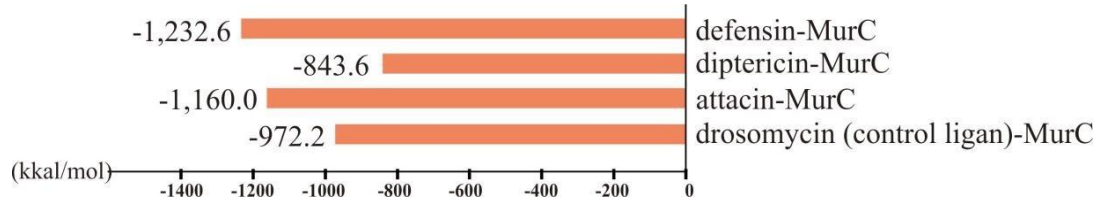

Fig. 4. Binding Affinity of AMPs Ligans with murC Receptor Used Cluspro 2.0

The results showed that defensin has a better binding affinity than control ligand (drosomycin) against murC, thus proving that defensin is an inhibitor capable of binding to the substrate. Binding affinity is the strength of the binding interaction between a single biomolecule (e.g. protein) to its ligand/binding partner. The more negative the binding affinity value, the easier it is to bind and can become a strong inhibitor [12]. The results of the docking analysis were then visualized using KFC 2 Server to determine which amino acid residues interact with each other, seen from the hotspot and the prediction score. The

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results showed that defensin, diptericin, and attacin with the receptor had similarities with the control ligand amino acid residues (drosomycin) with the receptor (**Table 2**).

Table 2. KFC 2 Visualized Result of Amino Acid Residues Interactions

Ligand- Receptor Binding	Amino Acids	Similarity with Control Ligand
Defensin-murC	Met:110, Arg:116, Phe:222, Arg:244, Ile:114, Phe:117, His:119, Met:187, Leu:225, Tyr:223, Glu:305, Glu:306	41,6%
Diptericin-murC	Arg:116, Phe:222, Arg:244	25%
Attacin-murC	Arg:3, Tyr:21, Leu:22, Met:110, Glu:113, Arg:1 16, Phe:117, Phe:222, Arg:244, Tyr:43, Arg:168, Tyr:169, Phe:222	41,5%
Control Ligand (Droso mycin)	Arg:6, Trp:14, Val:22, Glu:25, Met:110, Arg:116, Phe:117, Phe:222, Arg:244, Arg:168, Tyr:169, Tyr:223	100%

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p-ISSN: 2502-826X / e-ISSN: 2503-1163
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4. Result of Ligands Bioavailability and Toxicity Analysis

The results (**Table 3**) shows that almost all maggot extract compounds do not exceed Lipinski rules, except tannins and AMP (defensins). Lipinski is a rule used to determine the permeability and solubility of a compound to be used as an oral drug candidate. The Lipinski rule states that a compound has a good permeability or permeation level if it has a hydrogen bond donor <5, a hydrogen bond acceptor <10, a molecular weight <5, and a molar refractivity between 30-140. Based on Lipinski's Rule of Five, compounds that exceed more than two parameters are predicted to have low permeability or permeation level so that it will be difficult for the body to absorb as an oral drug candidate [13]. Because of the discovery of 2 compounds that exceed Lipinski rules, maggot extract have the good bioavailability result because do not exceed more than 3 rules of Lipinski.

Table 3. The Results of the Ligand Bioavailability Test Used Swissadme

Compund	Molecular weight (Da	H^+ Donor	H^- Acceptor	LogP	Molar reactivity
α-tocopherol	430.71	1	2	8.84	139.27
α-tocotrienol	424.66	1	2	8.6	137.85
β-sitosterol	414.71	1	1	8.02	133.23
Chittin	221.21	5	6	-3.08	47.19
Adipic acid	146.14	2	4	0.72	34.5
Tannins	636.47	11	18	-0.28	142.86
β-tocopherol	416.68	1	2	8.53	134.31
γ-tocopherol	416.68	1	2	8.53	134.31
β-tocotrienol	410.63	1	2	8.29	132.88
γ-tocotrienol	410.63	1	2	8.29	132.88
Defensin	34442.03	49	43	-19.98	990.27

Noted: the marked results are parameters that exceed the Lipinski rule

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The toxicity prediction with three parameters, namely inhibition of Human Ether-A-Go-Go-Related Gene (herG), carcinogenicity, and acute oral toxicity. The results show that all the test ligands are classified as weak inhibitors and are non-carcinogenic (**Table 4**). The results of the acute oral toxicity prediction also showed that all ligands passed the toxicity test and were in various categories. According to the US EPA (2018), category I has a value of LD50≤ 50mg / kg, category II has a value of 50 <LD50≤500 mg / kg, category III has a value of 500 <LD50≤5000 mg / kg, while category IV has a value of LD50> 5000 mg / kg.

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Table 4. The Results of The Ligands Toxicity Test Used Admestar 1.0

Compund	Parameters					
	HerG Inhibiton Toxicity		Carcinogenic		Acute Oral	
	Score	Category	Score	Category	Score	Category *
α-tocopherol	0.7192	Weak Inhibitor	0.8813	Non-carcinogenic	139.27	III
α-tocotrienol	0.5479	Weak Inhibitor	0.9184	Non-carcinogenic	137.85	III
β-sitosterol	0.8027	Weak Inhibitor	0.9182	Non-carcinogenic	133.23	IV
Chittin	0.9898	Weak Inhibitor	0.9735	Non-carcinogenic	47.19	IV
Adipic acid	0.9535	Weak Inhibitor	0.8307	Non-carcinogenic	34.5	IV
Tannins	0.9668	Weak Inhibitor	0.9614	Non-carcinogenic	142.86	III
β-tocopherol	0.7192	Weak Inhibitor	0.8813	Non-carcinogenic	134.31	III
γ-tocopherol	0.7192	Weak Inhibitor	0.8813	Non-carcinogenic	134.31	III
β-tocotrienol	0.5479	Weak Inhibitor	0.9184	Non-carcinogenic	132.88	III
γ-tocotrienol	0.5479	Weak Inhibitor	0.9184	Non-carcinogenic	132.88	III
Defensin	0.8904	Weak Inhibitor	0.7774	Non-carcinogenic	990.27	III

(*)Noted: Caterogy I: Highly toxic, Category II: Moderatelly toxic, Category III: Slightly toxic, Category IV: Practically non-toxic [14]

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IV. CONCLUSIONS

All bioactive compounds including the active peptides in the maggot *Hermetia illucens* are capable of acting as antibacterial *Streptococcus pneumonia*e which is the main co-infection of COVID-19 through inhibition of cell wall synthesis. Further in vitro studies are necessary to determine the MIC and MBC values, and in vivo test using acute respiratory infection mice model.

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