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Potential active compounds of *Streptomyces sennicomposti* GMY01 for antiplasmodial and antiSARS-CoV-2 revealed by targeted metabolomic and molecular docking

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ABSTRACT

Streptomyces sennicomposti GMY01 is a bacterium with huge biotechnological potential that revealed by genome mining analysis. This research aimed to investigate the potential compounds as antiplasmodial and the antiSARS-CoV-2 from the *S. sennicomposti* GMY01 using targeted metabolomic and *in silico* molecular docking. The crude extract was obtained by extraction of supernatant from fermentation product of the *S. sennicomposti* GMY01. The secondary metabolite profiling was obtained by using ultra-high-performance liquid chromatography (UHPLC) coupled to targeted high-performance mass spectrometry (HRMS) based on genome mining data of whole genome sequence (WGS). *In silico* molecular docking was performed on important target protein of *P. falciparum* i.e. glutathione reductase (PfGR), lactate dehydrogenase (PfLDH), phosphoethanolamine methyltransferase (Pfpmt), erythrocyte membrane protein 1 (PfEMP1) and glutathione-S-transferase (PfGST); and of SARS-CoV-2 proteins i.e. protease domain, spike glycoprotein, receptor-binding domain angiotensin-converting enzyme 2 (RBD-ACE2), 3-chymotrypsin-like protease (3CLpro), and RNA-dependent RNA polymerase (RdRp). One compound from *S. sennicomposti* GMY01 extract, albaflavenone was confirmed by targeted LC-HRMS. On molecular docking analysis, albaflavenone showed higher affinity than chloroquine as antiplasmodial drug and exhibited same affinity to remdesivir as antiSARS-CoV-2. *Stertomyces sennicomposti* GMY01 has promising biotechnological potential for drug development as antiplasmodial and anti-SARS-CoV-2 agent. Further study is needed, especially regarding *in vitro* testing of albaflavenone as antiplasmodial and antiSARS-CoV2.

ABSTRACT

Streptomyces sennicomposti GMY01 adalah bakteri dengan potensi bioteknologi yang besar berdasarkan hasil analisis penambangan genom. Penelitian ini bertujuan untuk mengetahui senyawa potensial sebagai antiplasmodium dan antiSARS-CoV-2 dari *S. sennicomposti* GMY01 menggunakan metabolomik tertarget dan doking molekuler. Ekstrak kasar diperoleh dari ekstraksi supernatan produk fermentasi *S. sennicomposti* GMY01. Profil metabolit sekunder diperoleh dengan menggunakan ultra kromatografi cair kinerja tinggi (UHPLC) digabungkan dengan spektrometri massa kinerja tinggi (HRMS) tertarget berdasarkan data penambangan genom dari seluruh urutan genom. Doking molekuler dilakukan pada protein target penting pada *P. Falciparum* yaitu *glutathione reductase* (PfGR), *lactate dehydrogenase* (PfLDH), *phosphoethanolamine methyltransferase* (Pfpmt), *erythrocyte membrane protein 1* (PfEMP1) dan *glutathione-S-transferase* (PfGST); dan protein SARS-CoV-2 yaitu *protease domain, spike glycoprotein, receptor-binding domain angiotensin-converting enzyme 2* (RBD-ACE2), *3-chymotrypsin-like protease* (3CLpro), dan *RNA-dependent RNA polymerase* (RdRp). Salah satu senyawa dari ekstrak *S. sennicomposti* GMY01, albaflavenone, terkonfirmasi oleh

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LC-HRMS tertarget. Pada analisis doking molekuler, albaflavenone menunjukkan afinitas yang lebih tinggi daripada klorokuin sebagai obat antiplasmodial dan menunjukkan afinitas yang sama terhadap remdesivir seperti obat SARS-CoV-2. Dengan demikian, *S. sennicomposti* GMY01 memiliki potensi bioteknologi yang menjanjikan untuk pengembangan obat sebagai agen antiplasmodial dan antiSARS-CoV-2. Penelitian lanjut diperlukan khusus nya uji *in vitro* albaflavenone sebagai antiplasmodial and antiSARS-CoV2.

INTRODUCTION

Severe acute respiratory syndrome corona virus-19 (SARS-CoV-2) has become a world pandemic that affected 213 countries around the world and infected more than 704 million people, with the number of confirmed deaths reaching more than 7 million people worldwide.[1] Unlike the previous coronavirus, SARS-CoV-19 has resulted in more deaths due to multiple organ dysfunction syndrome than to respiratory failure. This may be caused by the widespread expression of the angiotensin 2 modifying enzyme—the functional receptor for SARS-CoV-2—in multiple organs.[2] Based on the epidemiological update by the WHO, five SARS-CoV-2 VOCs have been identified since the beginning of the pandemic i.e. alpha (B.1.1.7), Beta (B.1.351), gamma (P.1), delta (B.1.617.2) and omicron (B.1.1.529. A variety of therapeutic options for SARS-CoV-2 are available that include antiviral drugs (e.g., molnupiravir, paxlovid, remdesivir), monoclonal antibodies, anti-inflammatory drugs, immunomodulators agents.[3]

The drugs recommended for COVID19 treatment include antiviral, antimalarial, antiparasitic, antibiotic, antifungal, anti-inflammatory, and other agents.[4,5] Chloroquine and its derivate hydroxychloroquine have been widely used as an alternative COVID-19 therapy,[6-8] although their safety level awaits evaluation.[9] Even though globally new cases of COVID19 have decreased by 58% and the death rate by 31% in 2024[10] vigilance regarding the emergence of new SARS cases must be anticipated. For COVID-19 drug development, a molecular docking approach on the target protein was one of the accelerated methods employed. Important targets, such as spike proteins on the surface of the coronavirus, receptor-binding domain angiotensin-converting enzyme 2 (RBD ACE-2), 3-chymotrypsin-like protease (3CLpro), RNA-dependent RNA polymerase (RdRp), and protease, were studied as protein targets for COVID-19 drug development.[11-15]

Bacteria of the genus *Streptomyces* are the main source of medicinal compounds for nearly 80% of drugs, especially antibiotics.[16] In our previous research, we discovered the marine bacterium *Streptomyces sennicomposti* GMY01, which has very high potential for development as a therapeutic agent. The bacterial extract shows anticancer activities on cancer cell lines.[17] and anti-plasmodium.[18] The results of a genome mining study found that *Streptomyces* sp. GMY01 revealed abundant biosynthetic gene clusters encoding secondary metabolite.[19]

In this study, we reported the metabolite profile of *S. sennicomposti* GMY01 extract by using targeted LC-HRMS and also performed molecular docking of detected active compounds against important protein targets on *P. falciparum* and SARS-CoV-2. Chloroquine and remdesivir, well known as antiplasmodium and anti COVID-19 were used as references.[7,20-22]

MATERIAL AND METHODS

Biological materials

Marine actinobacteria *S. sennicomposti* GMY01 were isolated from a marine sediment sample collected from Krakal Beach (8°8'44"S 110°35'59"E), Gunungkidul, Yogyakarta, Indonesia. *Streptomyces sennicomposti* GMY01 was deposited in the Indonesian Culture Collection (WDCM 769), Indonesian Institute of Sciences as InaCC A147 and NITE Biological Research Center (NBRC, WDCM 825), Japan with registration number NBRC 110111. The whole genome shotgun project of *Streptomyces* sp. GMY01 has been deposited at DNA Data Bank of Japan/ European Nucleotide Archive with GenBank accession number JABBNA000000000.[19]

Fermentation and extraction

Streptomyces sennicomposti GMY01 bacteria were maintained in International *Streptomyces* Project-2 (ISP-2) agar medium (Difco, Sparks, USA). It was cultured using liquid fermentation using starch nitrate broth (SNB) medium and extraction of secondary metabolites was conducted based on previous study.[18]

Targeted liquid chromatography -high resolution mass spectrometry (LC-HRMS)

Metabolomic analysis of the *S. sennicomposti* GMY01 extract was carried out using a ultra-high-performance liquid chromatography (TS Vanquish UHPLC) coupled to targeted high-performance mass spectrometry (HRMS) (Thermo Scientific Dionex Ultimate 3000 RSLC Nano UHPLC paired with Thermo Scientific Q Exactive) (Thermo Fisher Scientific, Bremen, Germany). Targeted UHLC-HRMS was based on a predicted compound formula which was obtained from the genome mining analysis of *Streptomyces* sp. GMY01 whole-genome sequence using antiSMASH version 6, available online at https://antismash.secondarymetabolites.org/. HRMS was carried out with mobile phases A (water + 0.1% formic acid) and B (acetonitrile + 0.1% formic acid). The column used was a Accucore™ Phenyl Hexyl analytical column (2.1 mm × 2.6 µm) (Thermo Fisher Scientific) with a flow rate of 40 µL/min, an injection volume of 5 µL, and a gradient with an analysis time of 25 min. The gradient was programmed as follows: 2 min, 5% B; 15 min, 60% B; 22 min, 95% B; 25 min, 95% B; 25.1 min, 5% B; and 30 min, 5% B. Experiments were carried out in full MS data dependent MSMS at 70,000 the full width at half maximum (FWHF) resolution, heated electrospray ionization, positive ionization, and data processing with Thermo Scientific XCalibur and Compound Discover 3.2.[23]

In silico molecular docking studies

Molecular docking was used to predict the binding affinity of several detected compounds of *S. sennicomposti* GMY01 with proteins that play a role in inhibiting the development of *P. falciparum* and SARS-CoV-2. The predicted compound was analysis using targeted UHPLC-HRMS) based on genome mining study. The structure of the compounds was created by using the ChemDraw online program (available online https://chemdrawdirect.perkinelmer.cloud/js/sample/index.html) based on the IUPAC name of the compound in the PubChem database (available online https://pubchem.ncbi.nlm.nih.gov/sources). This study used target proteins of *Plasmodium*: glutathione reductase (PDB ID: 1ONF), lactate dehydrogenase (PDB ID: 1CET), phosphoethanolamine methyltransferase (PDB ID: 4FGZ), erythrocyte membrane protein 1 (PDB ID: 3CPZ) and glutathione-S-Transferase (PDB ID: 4ZXG) and SARS-COV-2 protein: protease (PDB ID: 6LU7), spike protein

(PDB ID: 6LXT), receptor-binding domains (RBD ACE2) (PDB ID: 6VW1), 3-chymotrypsin-like protease (3CLpro) (PDB ID: 6M2N), and RNA-dependent RNA polymerase (RdRp) (PDB ID: 6M71), which were obtained from RCSB database. The 2D structure of compounds were retrieved from the ChemSpider webserver and converted to an optimized 3D structure using Marvinsketch tools (https://www.chemaxon.com). Both proteins and compounds were prepared using auto dock tools. The proteins were cleaned of water, ions, and other small molecules. Hydrogen atoms were added to polar groups of proteins to minimize errors caused by ionization and tautomeric states of amino acid residues. Molecular docking was performed on Autodock Vina,[24] while visualization of binding interactions was performed using the DisCoVery studio visualizer (DS visualizer) tool.[25] The computational simulation was carried out on a Windows 10 Operating system, with an AMD A8 7410 (Quad-core; 2.2 GHz) processor and 4 GB of RAM. The molecular docking study was observed from the affinity (kcal/mol) value with a root mean square deviation score less than 2 A, which expressed a visualization of the binding interaction between compounds and the active site of proteins.

RESULTS

Genome mining analysis of whole genome

Genome mining analysis of whole genome *Streptomyces* sp. GMY01 using antiSMASH version 6 revealed 28 region of biosynthesis gene clusters (BGCs) which 5 – 100% of similarity with most similar known cluster (TABLE 1). NRPS, PKS and hybrid of NRPS-PKS were dominant BGCs. Nine BGCs were NRPS type. Geosmin, ectoine, albaflavenone and informatipeptine were compounds as known cluster which has 100% similarity.

Metabolomic analysis of *S. sennicomposti* GMY01 extract

Targeted metabolomic analysis using UHPLC-HRMS based on genome mining analysis showed in TABLE 2. The target compound is the compound predicted to be produced by GMY01 based on genome mining analysis from whole genome sequence data.

There were 13 compounds detected using targeted LC-HRMS. Based on MS analysis, it is known that only one compound has the same formula and molecular weight, namely albaflavenone ($C_{15}H_{22}O$, 218.16672 Da of molecular weight). The albaflavenone spectra and ionization showed in FIGURE 1. Other compounds were detected to have changed the composition of the parent compound so that they had differences in formula and molecular weight.

In silico molecular docking on *Plasmodium* and SARS-Cov-2 proteins

Molecular docking of albaflavenone on the target protein of *Plasmodium* and SARS-CoV-2 showed TABLE 3 and TABLE 4. Albaflavenone has higher affinity than chloroquine as antimalarial. Therefore, the albaflavenone compound produced by *S. sennicomposti* GMY01 is expected to be an alternative antiplasmodium.

TABLE 1. Genome mining of whole genome *S. sennicomposti* GMY01 (NCBI accession number: JABBNA000000000) using antiSMASH version 6

Region	Type	From	To	Most similar known cluster	Similarity (%)
4.1	NRPS	1	2.456		
13.1	NRPS, lanthipeptide	31.245	98.413	Lysocin (NRP)	9
21.2	T3PKS	142.812	183.870	Herboxidiene (polyketide)	6
21.2	NRPS	323.141	366.443	Stenothricin (NRP,cyclic depsipeptide)	13
23.1	NRPS	1	23.556	Saframycin A/saframycin B (NRP)	12
24.1	Terpene	56.186	78.612	Geosmin (terpene)	100
24.2	Bacterocin, NRPS	94.123	145.168	Streptobactin (NRP)_	47
24.3	T1PKS, NRPS-like, NRPS, butyrolactone	203.147	325.428	Microansamycin (polyketide)	67
24.4	Siderophore	402.319	414.289		
25.1	Ectoine	249.254	259.658	Ectoine (other)	100
26.1	NRPS	46.922	95.389	Deimino-antipain (NRP)	66
27.1	lanthipeptide	88.365	111.274	Venezuelin (RiPP-Lanthipeptide)	50
27.2	Bacteriocin	155.537	166.355		
27.3	NRPS, other	179.507	271.021	S56-p1 (NRP)	17
28.1	NRPS	37.930	106.052	Mirubactin (NRP)	50
29.1	T1PKS, NRPS	1	73.488	Scabichelin (NRP)	90
30.1	Lanthipeptide	23.506	46.109		
36.1	Terpene	201.344	222.357	Albaflavenone (terpene)	100
38.1	Siderophore	30.127	43.346	Grincamycin (polyketide Type II + saccharide:hybrid+tailoring)	5
42.1	Butyrolactone	152.991	163.929	Scleric acid (NRP)	23
45.1	Terpene	7.057	30.222	Isorenieratene (terpene)	75
48.1	NRPS	1	16.929	Naphthyridnomycin (NRP)	17
49.1	Terpene	1	18.520	Hopene (terpene)	53
53.1	HgIE-KS, T1PKS	1	39.162	(2S,6R)-diamino-(5R,7)-dihydroxy-heptanoic acid (NRP)	24
53.2	Bacteriocin, lanthip-eptide	131.373	164.299	Informatipeptine (RiPP:Lanthipeptide)	100
55.1	Butyrolactone	68.670	79.605		
60.1	T1PKS	21.438	84.031	Abyssomicin M-X	56
63.1	T2PKS	51.542	124.087	Spore pigment	83

TABLE 2. Metabolite constituents detected from *S. sennicomposti* GMY01 extract using targeted UHPLC-HRMS)

Formula	Parent Compound	Transformations	Composition Change	Calc. MW	RT [min]	Area (Max.)
$C_{15}H_{22}O$	Albaflavenone	No change		218.16672	8.721	31373832.89
$C_{27}H_{46}N_8O_9$	Deimino-antipain	Hydration, Oxidative Deamination to Alcohol, Reduction	-(N) +(H_3O_2)	626.34087	10.53	11621202.08
$C_6H_{11}NO$	Ectoin	Nitro Reduction, Oxidative Deamination to Alcohol	-(NO) +(H)	113.08382	8.322	5844151.095
$C_{12}H_{22}O_2$	Geosmin	Oxidation	+(O)	198.16166	14.535	39413439.42
$C_{12}H_{22}O_3$	Grincamycin	Nitro Reduction, Reduction	-($C_{37}H_{40}O_{15}$)	214.15647	7.272	29666745.56
$C_{24}H_{42}O_4$	Herboxidiene	Nitro Reduction, Reduction	-(C O2)	394.30722	16.547	22234194.86
$C_{30}H_{48}O_2$	Hopene	Desaturation, Oxidation, Oxidation	-(H_2) +(O_2)	440.36409	16.439	3976220.706
$C_{17}H_{23}NO$	Microansamycin	Desaturation, Nitro Reduction, Nitro Reduction	-(O_4) +(H_2)	257.17781	8.99	7401282.485
$C_{19}H_{30}N_6O_8$	Mirubactin	Reduction	-($C_7H_2O_3$)	470.2112	12.909	5343696.902
$C_{21}H_{20}N_2O_5$	Naphthyridinomycin	Dehydration, Dehydration, Oxidative Deamination to Ketone	-(H_7NO)	380.13652	7.762	14737821.53
$C_{16}H_{24}N_4O_9S$	s56-p1	Nitro Reduction, Oxidative Deamination to Alcohol, Oxidative Deamination to Ketone	-(H_2N_2)	448.12724	7.034	7952669.364
$C_{28}H_{32}N_4O_4$	Saframycin A	Nitro Reduction, Nitro Reduction	-(CO_4) +(H_2)	488.2412	5.629	11451413.8
$C_{28}H_{34}N_2O_8$	Saframycin B	Hydration, Nitro Reduction, Oxidative Deamination to Alcohol	-(N) +(H_3)	526.23002	6.288	181103280.2

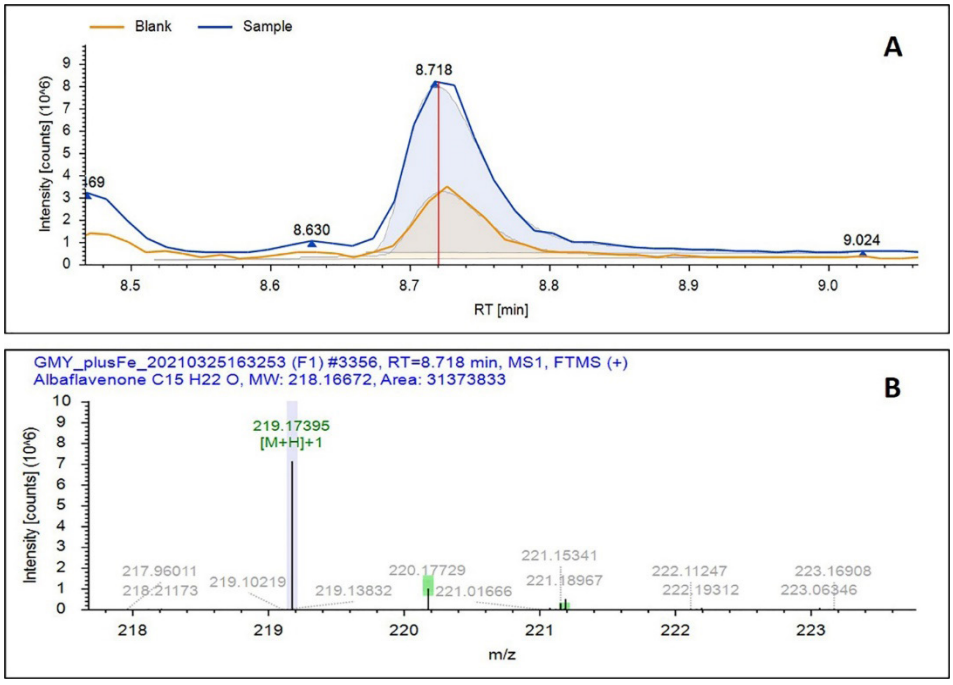

FIGURE 1. Spectra and ionization of albaflavenone in targeted analysis of LC-HRMS)

TABLE 3. Docking score of detected compounds in *S. sennicomposti* GMY01 on five potential binding domains of *P. falciparum*

Compounds	Affinity [energy gibbs (kcal mol^{-1})]				
	1ONF	1CET	4FGZ	3CPZ	4ZXG
Hopene	-9.0	-8.8	-7.9	-6.9	-9.2
Albaflavenone	-7.1	-5.7	-6.3	-6.8	-6.3
Geosmin	-5.8	-5.4	-5.6	-6.3	-5.9
Isorenieratene	-9.3	-8.1	-9.0	-7.5	-8.4
Grincamycin	-11.7	-12.6	-11.8	-9.6	-11.6
Vazabitide A	-6.5	-5.3	-4.7	-5.9	-5.2
Herboxidiene	-6.8	-6.8	-6.2	-5.8	-6.5
Saframycin A	-7.2	-7.0	-8.5	-6.6	-
Chloroquine*	-5.1	-5.0	-4.9	-5.4	-5.5
Remdesivir*	-6.8	-7.1	-6.8	-6.0	-7.1

Note: *reference compounds; Glutathione reductase (1ONF); Lactate dehydrogenase (1CET); Phosphoethanolamine methyltransferase (4FGZ); Erythrocyte membrane protein 1 (3CPZ); Glutathione-S-transferase (4ZXG)

TABLE 4. Docking scores of detected compounds in *S. sennicomposti* GMY01 extract toward several potential binding domain of SARS-CoV-2

Compounds	Affinity [Energy gibbs (kcal mol^{-1})]				
	6LU)	6LXT	6VW1	6M2N	6M71
Hopene	-7.4	-8.6	-8.8	-8.1	-8.1
Albaflavenone	-6.3	-6.3	-6.0	-6.8	-6.0
Geosmin	-5.4	-5.3	-5.6	-5.7	-5.5
Isorenieratene	-7.6	-7.4	–8.1	-7.8	-8.5
Grincamycin	-10.8	-11.9	-12.0	-12.0	-11.4
Vazabitide A	-5.5	-5.1	-5.4	-5.7	-5.3
Herboxidiene	-5.7	-6.0	-6.8	-6.2	-6.8
Saframycin A	-6.4	-7.6	-7.9	-8.7	-7.3
Chloroquine*	-4.9	-5.2	-5.0	-5.9	-4.8
Remdesivir*	-6.2	-6.9	-6.7	-6.5	-6.4
Hesperetin*	-6.7	-7.4	-7.3	-7.9	-7.3

Note: *reference compounds; Protease domain (6LU7); Spike glycoprotein (6LXT); RBD-ACE2 (6VW1); 3CL Pro (6M2N); RdRp (6M71)

A three-dimensional illustration of binding between albaflavenone and the target proteins of *Plasmodium* is shown in FIGURE 2. Albaflavenone has the highest affinity for gluthathione reductase and erythrocyte membrane protein-1 of *Plasmodium*.

Albaflavenone also showed the same affinity with remdesivir as the SARS-CoV-2 drugs. Chloroquine as anti-plasmodial drug have lower affinity than remdesivir as antiviral compound. A three-dimensional illustration of binding between albaflavenone and the target proteins of SARS-CoV-2 is shown in FIGURE 3. Albaflavenone has the highest affinity for 3-chymotrypsin-like protease (3CL pro) and the lowest for angiotensin-converting enzyme 2 (RBD ACE2) and RNA-dependent RNA polymerase (RdRp) in target proteins of SAR-CoV-2.

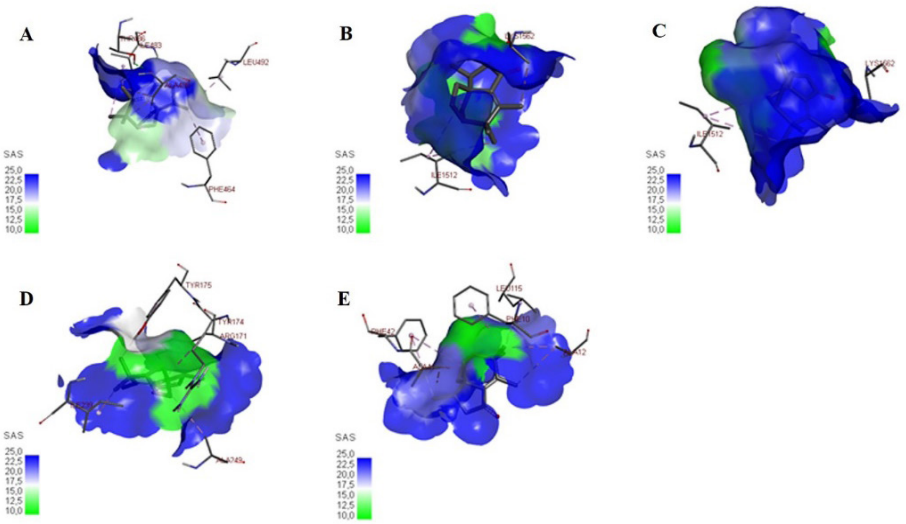

FIGURE 2. Three dimensional of docking visualization of albaflavenone on *P. falciparum* proteins: A) glutathione reductase (1ONF); B) erythrocyte membrane protein 1 (3CPZ); C) phosphoethanolamine methyltransferase (4FGZ); D) lactate dehydrogenase (1CET); and E) glutathione-S-transferase (4ZXG).

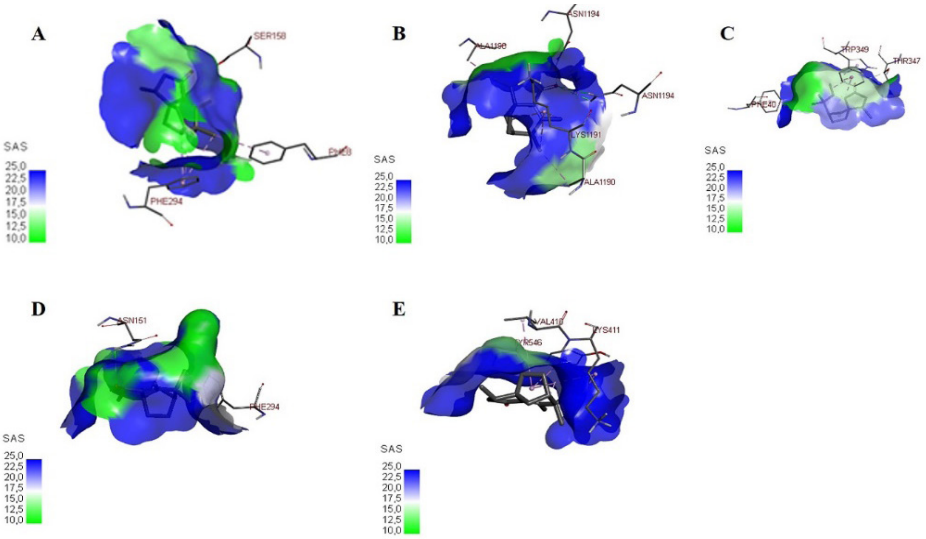

FIGURE 3. Three dimensional of docking visualization of albaflavenone on domain of SARS-CoV-2:A) protease domain; B) spike glycoprotein; C), angiotensin-converting enzyme 2 (RBD-ACE2); D) 3-chymotrypsin-like protease (3CL Pro); and E) RNA-dependent RNA polymerase (RdRp).

DISCUSSION

Streptomyces sp. GMY01 has a large group of NRPS-encoded secondary metabolites (TABLE 1). This result was similar with another previous study demonstrating NRPS gene diversity in GMY01 based on PCR amplification and restriction fragment analysis of NRPS genes with *Hae* III to assess the diversity of NRPS genes.[26] Genome mining analysis using anti SMASH version 3 showed that strain GMY01 harboring 10 different NRPS gene clusters that encode secondary metabolites, as pure NRPS or hybrid between NRPS and other compounds.[26] These results indicate that the marine origin of *Streptomyces* has high potential to be developed as a very large source of activity. However, compared with others marine *Streptomyces*, which have almost the same genome size, the BGC in GMY01 is less than that in *Streptomyces* MP131-18.[27]

In the previous study, genome mining analysis of a whole-genome sequence of *S. sennicomposti* GMY01 revealed abundant gene clusters such as non-ribosomal peptide synthetases (NRPS), polyketide synthases (PKS), and hybrid NRPS-PKS, with sequence identities to the most similar known cluster range from 9% to 90%.[26] This indicates that *S. sennicomposti* GMY01 is a marine organism with biotechnological potential as a drug source. In a previous bioassay study, GMY01 exhibited anticancer activity against human breast carcinoma T47D cells (IC_{50} = 19 µg mL^{-1}) and MCF7 cells (IC_{50} = 7 µg mL^{-1}).[28] Therefore, this marine-derived organism has great potential as a new lead compound. Although the anti-plasmodial mechanism has not been studied in this research.

The results of targeted LC-HRMS demonstrated that albaflavenone was confirmed as secondary metabolites produced by *S. sennicomposti* GMY01 (FIGURE 2). Albaflavenone ($C_{15}H_{22}O$) was detected with no transformation and was no change formula with parent compound (based on genome mining) at 8.7 min of retention time. This result was similar with genome mining analysis (TABLE 1) which 100% similarity with known cluster. This compounds as known as sesquiterpene antibiotic and resulted by *S. coelicolor* A3(2).[29] The biosynthesis of albaflavenone in *S. coelicolor* clearly demonstrate that involves the coupled action of epi-isozizaene synthase and CYP170A1 which is encoded by the sco5222 and sco5223 gene.[29,30] The purified recombinant protein encoded by sco5222 was shown to catalyze the Mg^{2+}-dependent conversion of farnesyl diphosphate to epi-isozizaene. The sco5223gene encodes a cyctochrome P450 that catalyzes the oxidation of epi-isozizaene to albaflavenone. Stereo isomeric albaflavenols are intermediates in the oxidation process.[31]

In molecular docking, albaflavenone better affinity than chloroquine, which is known as antimalarial (TABLE 3). Albaflavenone has higher affinity (-5.7 to – 7.1 kcal.mol^{-1}) than chloroquine (- 4.9 to – 5.5 kcal.mol^{-1}) on all target proteins of *Plasmodium*. This indicated that albaflavenone from GMY01 has high potency for new antiplasmodial drug. Remdesivir as an antiviral also has a high affinity on *Plasmodium*. This indicates that this antiviral compound also has the potential to be used as an antiplasmodial. Although there is no previous study that proves this phenomenon. Albaflavenone has the highest affinity for gluthathione reductase (-7.1 kcal.mol^{-1}) and erythrocyte membrane protein-1 (-6.8 kcal.mol^{-1}) (TABLE 3**)**. In three-dimensional visualization shows that albaflavenone has bind amino acid valine (410), tyrosine (546), lysine (411) on glutathione reductase; lysine (1562), isoleucine (1512) on erythrocyte membrane protein-1; lysine (1562), isoleucine (1512) on phosphoethanolamine methyltransferase; isoleucine 9239), arginine 9171), tyrosine (175), tyrosine (174), alanine (249) on lactate dehydrogenase and Alanine (12), alanine (41), phenylalanine (42), phenylalanine (10), leucine (115) on

glutathione S-transferase (FIGURE 3). Glutathione is the most abundant low molecular weight redox active thiol in the parasites existing primarily in its reduced form representing an excellent thiol redox buffer. This allows for an efficient maintenance of the intracellular reducing environment of the parasite cytoplasm and its organelles.[32] Glutathione reductase (GR), which is a major antioxidant enzyme, is targeted in the treatment of many diseases due to the dual role of its product, reduced glutathione (GSH), in infected cells.[33] Approximately 60 different *P. falciparum* erythrocyte membrane protein 1 (PfEMP1) variants are encoded by each haploid genome of *P. falciparum*. Such *P. falciparum* CM parasites express a subgroup of group A PfEMP1s that facilitates dual binding to host intercellular adhesion molecule-1 (ICAM-1) and endothelial protein C receptor EPCR.[34] The high affinity of albaflavenone compound to these two proteins is predicted to inhibit the growth of *P. falciparum*.

Among all target proteins of SARS-CoV-2, albaflavenone has affinity (-6 to -6.8 kcal mol^{-1}) for protease domain, spike glycoprotein, RBD-ACE2, 3CLpro and RdRp as important targets on the SARS-CoV-2 virus (TABLE 3). In three-dimensional visualization shows that albaflavenone has bind amino acid Serin (158), phenylalanin (8), phenylalanin (294) on protease domain; asparagine (1194), alanin (1190), lysin (1191) on spike glycoprotein; threonine (347), tryptophan (349), phenylalanine (40) on RBD-ACE2; asparagine (151), phenylalanine (294) on 3CL-Pro and valine (410), tyrosine (546), lysine (411) on RdRp (FIGURE 4). This affinity almost the same as affinity of remdesivir (-6.2 to -6.9 kcal mol^{-1}) as COVID-19 drug. This indicates that the albaflavenon produced by GMY-01 has the potential to become a COVID-19 drug. Spike protein and 3CLpro are very important for the transmission and virulence of the SARS-CoV-2 virus.[11] ACE2 molecule was known as a human entry receptor for spike which fasilitates its cross-species transmission. Recent research speculated that SARS-Spike-reseptor binding domain (RBD) also bind ACE2 and resulted a complex.[14] This is supported by the receptor-binding motif analysis which showed that RBD make direct contact with ACE2.[12] RNA polymerase that depends on RNA (RdRp) is a vital protein in the replication / transcription process of the corona virus. In research on SARS-CoV inhibitor compounds, RdRp is widely used as a very important drug target.[14] By inhibiting one or more of these five proteins for active treatment and therapy, the severity of viral infection is expected to be reduced. Thus, similar to several other studies, the use of chloroquine as a COVID-19 drug needs to be evaluated. In other study, chloroquine antimalarial showed *in vitro* antiviral effect against SARS-CoV-2.[22]

CONCLUSION

This finding is important for further research, especially *in vitro* assay of albaflavenone on *P. facliparum* and the SARS-CoV-2 virus. The very low toxicity on normal cells also indicated that the marine-derived bacterium *S. sennicomposti* GMY01 has promising potency as a safe drug candidate for antiplasmodial, and anti-SARS-CoV-2. To the best of our knowledge, there have been no other scientific reports about albaflavenon as antiplasmodial, and antiSARS-CoV-2.

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