OPEN ACCESS
Journal of Multidisciplinary Applied Natural Science Vol. 5 No. 1 (2025) https://doi.org/10.47352/jmans.2774-3047.236
Check for updates
Research Article

Profiling and Antibacterial Activity Assay of Secondary Metabolites from *Streptomyces* Isolated from Mangrove Sediment Sample

Dwi Marwati Juli Siswanti, Kasta Gurning, Winarto Haryadi*, Chairil Anwar, and Respati Tri Swasono

Received : September 10, 2024	Revised : October 26, 2024	Accepted : October 28, 2024	Online : December 14, 2024

Abstract
Bacterial infections pose significant threats to human health, particularly due to the rise of antibiotic-resistant strains. Identifying new sources of effective antibiotics is therefore crucial for combating these resistant pathogens. This study aims to isolate novel *Streptomyces* species and profile their secondary metabolites through extraction and high-resolution mass spectrometry (HRMS) analysis. Furthermore, the antibacterial activity of the extract containing the secondary metabolites was assessed through the *in vitro* agar well diffusion method and supported by the molecular docking, molecular dynamics simulations, and drug-likeness analysis. Sediment samples were collected from mangrove forests in Yogyakarta. The bacteria were then isolated, purified, and characterized using 16S rRNA sequencing. The isolates were then cultured to enrich the secondary metabolites, and their secondary metabolites were extracted using methanol and dichloromethane solvents in a 1:1 volume ratio. The results showed that the isolated bacteria of *Streptomyces sp.* were obtained with a 95.44% similarity rate, which produced several secondary metabolites. The *in vitro* antibacterial assay of the extract resulted in an inhibition zone of 14, 14, and 15 mm against *Propionibacterium acnes*, *Staphylococcus aureus*, and *Escherichia coli*, respectively. The molecular docking and molecular dynamic simulations for 100 ns revealed that compound SB236057A could inhibit the function of thymidylate kinase protein through a carbon-hydrogen bond with Glu37 residue. Furthermore, drug-likeness analysis showed that the secondary metabolites of *Streptomyces sp.* exhibited preferable drug-likeness and pharmacokinetic properties. This research focuses on the understanding of microbial biodiversity in mangrove sediments, particularly focusing on the genus Streptomyces and its potential to produce novel antibiotics.

Keywords: mangrove, *Streptomyces*, antibiotic, *in silico*, *in vitro*, drug-likeness

1. INTRODUCTION

Mangroves are coastal intertidal areas with unique ecosystems that are often used for biochemistry exploration [1]. This unique ecosystem is mainly influenced by the salinity, which is affected by tides and climate, making the mangrove area different from the other areas. Mangrove forests are vital ecosystems that have not been fully explored yet, offering significant opportunities for discovering new species and potential resources. These forests are not only important for their ecological functions, such as protecting coastlines and serving as nurseries for marine life, but also have potential for medicines and industrial raw materials. Responsible exploration can help maintain its sustainability and explore its beneficial value for humans, especially for antibiotic purposes [2].

It was reported that the existing antibiotics are unable to deal with increasingly diverse bacteria called resistance cases. This crisis is caused by the overuse and misuse of antibiotics, as well as the lack of new drug development by the pharmaceutical industries. Bacteria can protect themselves and resist antiseptics in various ways, such as strengthening cell walls, forming a protective layer, destroying, or changing antiseptic targets, or increasing target production, so that they become more resistant to antimicrobial agents [3]. Therefore, it is challenging to extract and isolate new antibacterial agents from the bacteria.

Streptomyces is a Gram-positive bacterium that can produce various secondary metabolites. It was reported that different Streptomyces strains will produce different secondary metabolites, with different abilities or activities [4]. Secondary metabolites are natural molecules with diverse chemical structures and biological activities that play important roles in plants, including defense, attraction, communication, and stress mediation [4] [5]. Streptomyces species has been used as a source of new antibiotics because of its potential to

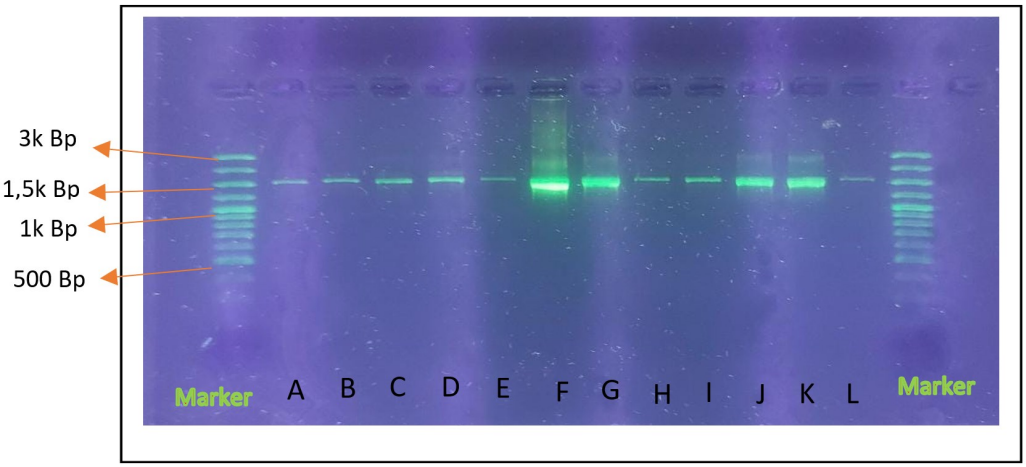

Figure 1. Visualization of PCR electrophoresis results of rRNA isolates A-L isolated from mangrove sediments.

produce compounds that have broad biological capabilities [6]. Almost two-thirds of all microbial antibiotics are initially sources in Streptomyces. Cephamycin, chloramphenicol, tetracycline, kanamycin, spectinomycin, monensin, and mitomycin C are derived from various species of Streptomyces, including *S. clavuligerus*, *S. venezuelae*, *S. aureofaciens*, *S. kanamyceticus*, *S. spectablis*, *S. cinnamonensis*, and *S. lavendulae*, respectively. On the other hand, *S. griseus*, *S. avermitilis*, and *S. cattleya* produce streptomycin, avermectin polypeptide, and fluorometabolites [3]. Moreover, some of the secondary metabolites from Streptomyces have been used as lead compounds in the pharmaceutical and agricultural fields [7]-[9].

Streptomyces can be isolated from marine waters, soil, rocks, or even plants whereas the growth medium will significantly affect the secondary metabolites produced by an organism. However, current secondary metabolite profiling does not represent all Streptomyces found in several mangrove forests in Indonesia [10]-[12] New secondary metabolites are expected to be discovered from extreme or unique media, including mangrove sediments. According to Wang et al., Actinomycetes produced from the sea have a diversity of structures and bioactivity [13]. Therefore, it is expected that Streptomyces isolated from mangrove sediments will produce more diverse secondary metabolites compared to other territories [14]-[16].

One of the powerful analytical techniques to identify secondary metabolites is high-resolution mass spectrometry (HRMS) [6][17]. Further, *in vitro* and *in silico* tests can be used to examine the antibiotic activity. The common *in vitro* assay is conducted through agar well diffusion method while the *in silico* method employs a computer that is carried out to understand the mechanism of action of an antibacterial agent [18]. Molecular docking and molecular dynamics simulations are frequently used to predict the orientation of a ligand on the binding site of a protein receptor and assess the level and stability of the binding affinity [19]-[21]. All of this is done to increase the cost and time efficiency of research because it can speed up the process and increase the success rate [18].

This research aims to isolate Streptomyces from mangrove sediments, to create a profile of secondary metabolite compounds produced, and to evaluate the antibacterial activity through *in vitro* and *in silico* tests. This research is crucial as it can help discover new secondary metabolites with unique biological properties, understand the biodiversity of Streptomyces secondary metabolites in mangrove forests, develop new strategies for the exploration and exploitation of Streptomyces secondary metabolites, and study the ecology and evolution of Streptomyces secondary metabolites.

2. MATERIALS AND METHODS

2.1. Materials and Methods

Samples were obtained from mangrove forest sediments at Baros Beach, with a geographical location of 110°28'50° East Longitude and 7°

99'50° South Latitude, Bantul Regency, Special Region of Yogyakarta Province, Indonesia. The materials used include SCA media (Starch Casein Agar), International *Streptomyces* Project-2 Medium (ISP-2), Smobio DM2400 marker, methanol (Merck), dichloromethane (Merck), and *n* -hexane (Merck). The equipment used includes an autoclave, laminar airflow and shaker incubator, rotary vacuum evaporator, BigDye Terminator v3.1 kit (Applied Biosystems) and primers, an automated DNA sequencer (ABI PRISM 3130 Genetic Analyzer; Applied Biosystems), and high-resolution mass spectrometry (Thermo Scientific Q Exactive Hybrid Quadrupole-Orbitrap Mass Spectrometer). The research was conducted at the Microbiology Laboratory of the Center for Food and Nutrition Studies, Universitas Gadjah Mada, Indonesia.

2.2. Sample Preparation and Isolation

Sediment samples were taken and put into a coolbox, then dried using an oven at 60 °C. Drying aims to reduce non-Streptomyces organisms. Isolation of bacteria using starch casein agar (SCA) media with the pour plate technique follows previous research with slight modifications. As many as 20 samples that have been filled with media are incubated for 6 days at room temperature. After the incubation time is reached, bacterial colonies are selected based on color, shape, size, texture, and turbidity. Furthermore, the samples are isolated and purified to be further cultured and grown on broth media.

2.3. Identification and Characterization of Isolates

Bacterial isolates are identified and characterized based on colony morphology, color grouping, and Gram staining to ensure the uniformity of the types included in the genus Streptomyces. Uniform bacterial isolates are further analyzed with 16S sequencing to ensure that the isolates are included in the genus Streptomyces. The results of bacterial isolation from mangrove sediment were confirmed to be the genus Streptomyces. The isolates that were suspected to be pure, DNA were isolated and amplified by PCR (with a universal primer) according to the method used and analyzed using electrophoresis. The level of DNA similarity was also evaluated using the basic local alignment search tool (BLAST) program and then compared with nucleotide sequences stored in the GenBank database using National Center for Biotechnology Information BLAST.

2.4. Culture, Analysis, and Bioactivity Testing

Streptomyces culture was developed in starch nitrate broth (SNB) media in a shaker incubator with a growth rate of aerial mycelium at a temperature of 28 °C for 14 days. Harvesting was carried out aseptically in laminar airflow. Pellets and media were separated by centrifugation and

Table 1. Results of Isolation Sequence Alignment Analysis Using BLAST.

Isolate	Species name	Max score	Total score	E-value	Query cover	% Identity	Streptomyces category
A	*Streptomyces* sp. NXHG3	1888	1888	0.0	97%	90.59%	Yes
B	*Bacterium*	1781	1781	0.0	99%	96.80%	No
C	*Streptomyces castaneus*	1487	1487	0.0	92%	91.75%	Yes
D	*Enterobacter hormaechei*	2242	2242	0.0	98%	94.63%	No
E	*Streptomyces lannensis*	863	863	0.0	81%	81.51%	Yes
F	*Enterobacter hormaechei*	2455	2455	0.0	99%	97.03%	No
G	*Bacillus* sp. CCBAU 10759	2429	2429	0.0	99%	97.69%	No
H	*Streptomyces albogriseolus*	952	952	0.0	74%	84.4%	Yes
I	*Streptomyces* sp. OAct 128	2206	2206	0.0	99%	95.44%	Yes
J	*Bacterium*	2457	2457	0.0	99%	97.45%	No
K	*Staphylococcus* sp.	1886	1886	0.0	100%	97.55%	No
L	*Aeromonas sanarellii*	890	890	0.0	71%	84.68%	No

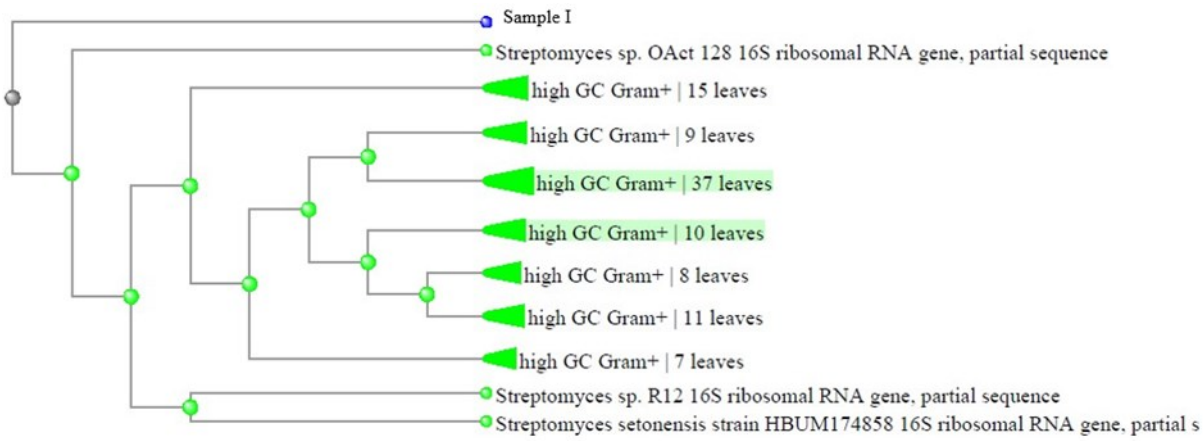

Figure 2. Phylogenic Tree bacterial isolate I results of sequencing analysis 16S.

filtration. The pellets were macerated with methanol, while the supernatant was extracted with methanol and dichloromethane in a ratio of 1:1. Methanol and dichloromethane represent polar and non-polar solvents; thus, both polar and non-polar compounds could be extracted. Furthermore, methanol and dichloromethane are miscible each other thus simplify the technical issues during the extraction process. The extract was then concentrated with a rotary evaporator before being analyzed by HRMS. In parallel, the extracts were also tested for in vitro bioactivity against *Staphylococcus aureus*, *Escherichia coli*, and *Propionibacterium acnes bacteria* by the agar diffusion method. The bioactivity testing procedure against bacteria followed previous studies with slight modifications [22]. HRMS data were analyzed by sorting mass adducts and their mass errors, namely the ratio between theoretical mass and observed mass. The compounds obtained were then analyzed in silico with molecular docking and drug-likeness prediction. Molecular docking was performed on the PDB ID 4QGH protein, taken from the www.rscb.org page. After initial treatment, using the Vina application. Analysis of secondary metabolites using the Analyzer facility owned by LPPT UGM is HRMS Q-orbitrap mode.

2.5. *Molecular Docking and Molecular Dynamics Simulations*

The chemical structures of the extracted compounds (**1**–**10**) are built by using Gaussian software. The native ligand, i.e., 2-(3-chlorophenoxy)-3-fluoro-4-{(1*S*)-3-methyl-1-[(3*S*)-3-(5-methyl-2,4-dioxo-3,4-dihydropyrimidin-1(2*H*)-yl)piperidin-1-yl]butyl}benzoic acid and compounds **1**–**10** were processed using YASARA software and saved in .pdb format. To prepare the structure files of the thymidylate kinase protein and its native ligand, water molecules, and other residues were removed. The simulation cell was defined by selecting the native ligand and creating a cube-shaped cell based on the Cartesian coordinate of the ligand. The native ligand was then removed and the protein structure with simulation cell was saved in .yob format. The molecular docking was performed using the VINA-based YASARA software according to the reported procedure [23]. Each individual compound was docked with 100 VINA runs and the docked structure was visualized with Discovery Studio software. The molecular dynamics simulations were performed for the most potential compound. The simulation was done for 100 ns with a pH of 7.4 employing AMBER14 forcefield at 37 °C following the previous method [23]. Each snapshot was analyzed to obtain the root mean square deviation (RMSD), root mean square fluctuation (RMSF), and hydrogen bond stability. Furthermore, the drug-likeness analysis was carried out using the SwissADMET online program (www.swissADMET.ch).

3. RESULTS AND DISCUSSIONS

3.1. *Sample Preparation, Isolation, and Identification of Isolates*

The genus Streptomyces isolates that were suspected to be pure, DNA were isolated and amplified by PCR (with a universal primer) according to the method used and analyzed using

Table 2. HRMS analysis of the isolate.

No	Compound name	Molecular formula	Retention time (min)	FMTS
1	NP-001798 or (*Z*)-*N*-(1-hydroxy-3-phenylpropan-2-yl)benzimidic acid	$C_{16}H_{17}NO_2$	8.53	+
2	Difenoconazole or 3-((2-(2-chloro-4-(4-chlorophenoxy)phenyl)-4-methyl-1,3-dioxolan-2-yl) methyl)-1*H*-1,2,4-triazole	$C_{19}H_{17}Cl_2N_3O_3$	16.96	+
3	SB236057A or (1'-ethyl-6,7-dihydro-2*H*,5*H*-spiro[furo[2,3-f]indole-3,4'-piperidin]-5-yl)(2'-methyl-4'-(5-methyl-1,3,4-oxadiazol-2-yl)-[1,1'-biphenyl]-4-yl)methanone	$C_{33}H_{34}N_4O_3$	20.09	+
4	Solanidine or (4*S*,6a*R*,6b*S*,8a*S*,8b*R*,9*S*,9a*R*,12*S*,14a*S*,15a*S*,15b*S*)-6a,8a,9,12-tetramethyl-3,4,5,6,6a,6b,7,8,8a,8b,9,9a,10,11,12,13,14a,15,15a,15b-icosahydro-1*H*-naphtho[2',1':4,5] indeno[1,2-b]indolizin-4-ol	$C_{27}H_{43}NO$	12.02	+
5	Daidzein or 7-hydroxy-3-(4-hydroxyphenyl)chroman-4-one	$C_{15}H_{10}O_4$	10.47	+
6	2-(Acetylamino)-3-(1*H*-indol-3-yl)propanoic acid	$C_{13}H_{14}N_2O_3$	9.01	-
7	NP-021797 or (*Z*)-3-hydroxydodec-5-enoic acid	$C_{12}H_{22}O_3$	14.28	-
8	NP-002089 or 5a-hydroxy-1,7,7-trimethyl-1,2,3,3a,5a,6,7,8-octahydrocyclopenta[c]pentalene-4-carboxylic acid	$C_{15}H_{22}O_3$	18.59	-
9	NP-008309 or (*R*,*Z*)-2-((1-hydroxyethylidene)amino)-3-phenylpropanoic acid	$C_{11}H_{13}NO_3$	8.47	-
10	Genistein or 5,7-dihydroxy-3-(4-hydroxyphenyl)-4*H*-chromen-4-one	$C_{15}H_{10}O_5$	11.87	-

electrophoresis so that a visualization of the band was obtained in Figure 1 [24]. The sample bands that are parallel or nearly parallel to the 1500 bp band, indicate that it is likely that all samples contain DNA fragments of about 1500 bp in size, as the target DNA was Streptomyces. In addition to the presence of the target band, there are not many faint bands or "smears", which can indicate the absence of contamination or DNA degradation. The intensity of the band can also provide clues about the efficiency of the DNA amplification process, PCR. The next step is BLAST which is used to obtain a level of similarity with existing Streptomyces bank data. Based on BLAST data, isolate I has similarities with *Streptomyces sp*. The results of the 16S rRNA analysis are acceptable if they have at least a >95% similarity rate [25]. Isolate I has a similarity rate of 95.44% with *Streptomyces* sp. Therefore, it can be ascertained that this isolate is a genus of Streptomyces.

Bacterial identification with the 16S rRNA sequencing method has several advantages, including faster and more accurate because it is not biased. In general, the process is passed through DNA extraction, amplification of the 16S region using PCR, and gene visualization by electrophysiology, sequencing, and processing the sequencing data with bioinformatics. The success of the gen extraction and amplification of the 16S region can be seen from the visualization of the

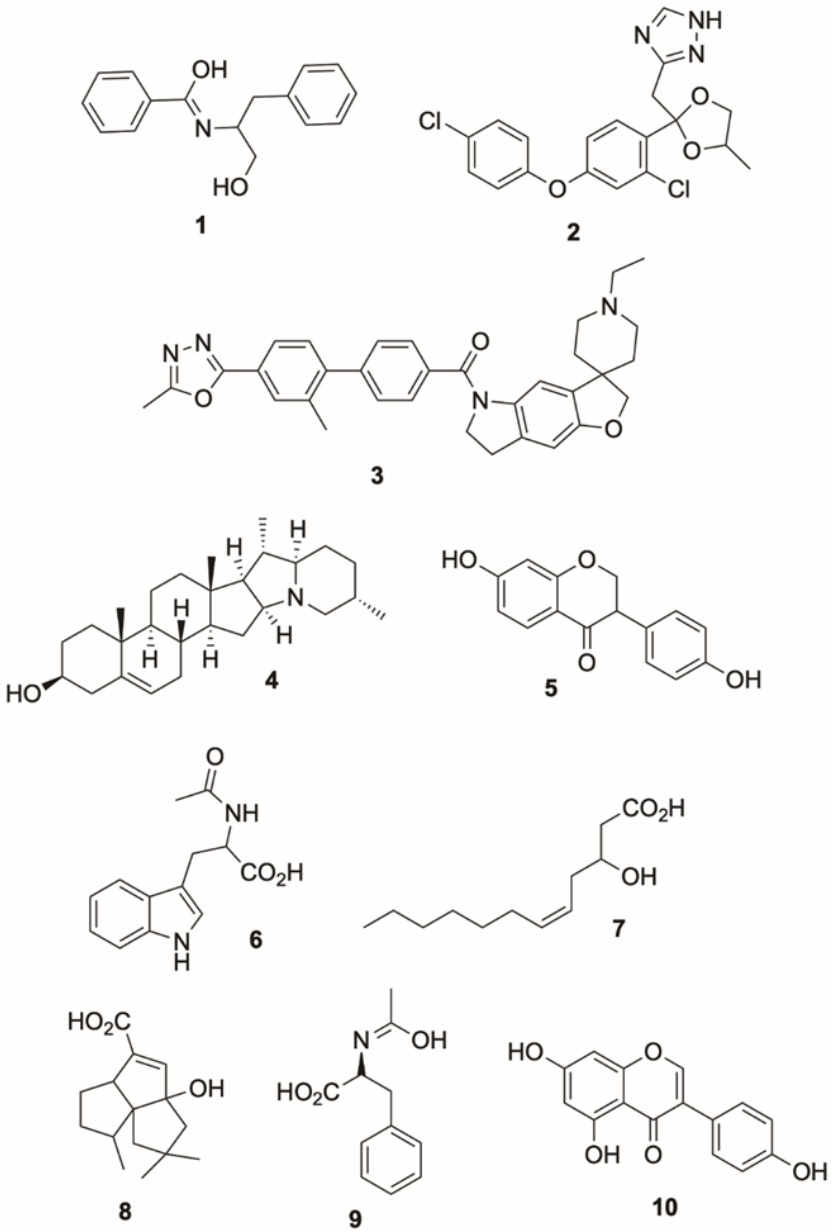

Figure 3. The chemical structures of the extracted compounds from the isolate.

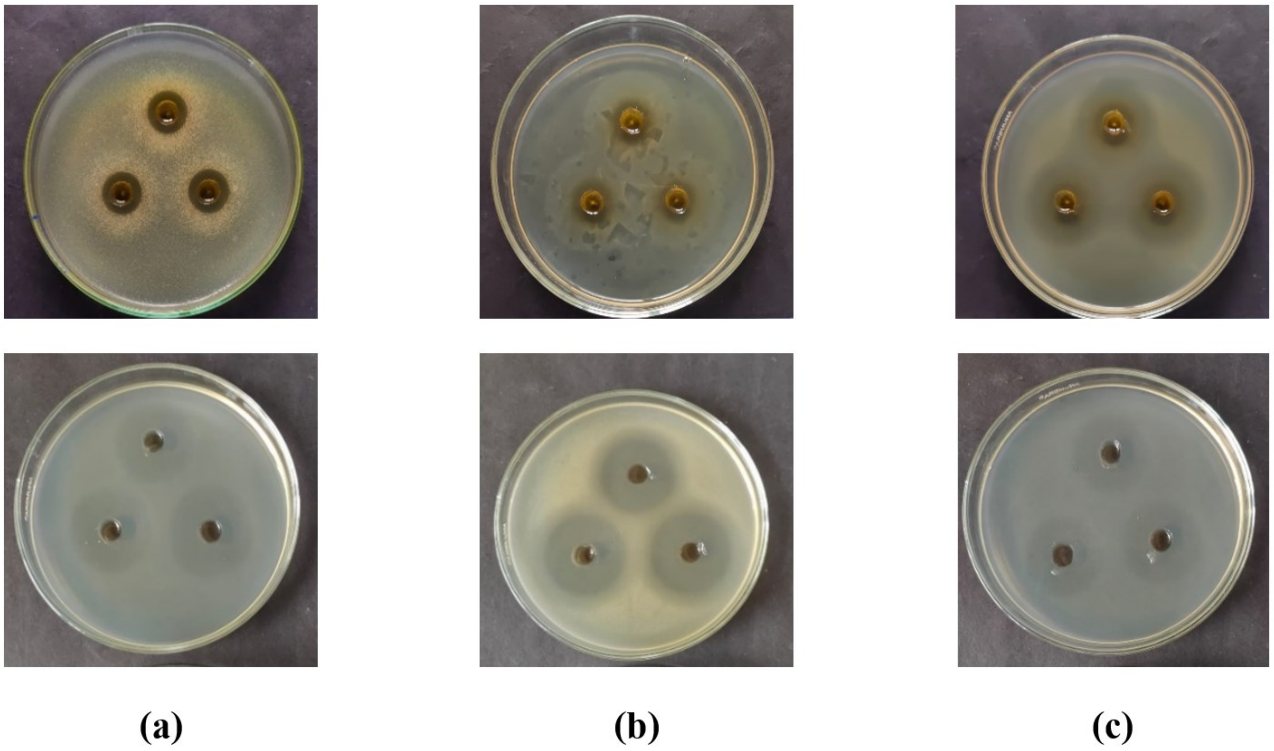

Figure 4. Results of *in vitro* test of streptomyces extract (first row) and chloramphenicol (second row) against (a) *P. acne*, (b) *S. aureus*, and (c) *E. coli* bacteria.

electrophoresis results in Figure 1. It can be seen that the band is quite thick and not shaded, indicating that the gene extraction results have been successful. The sequencing results were compared with bank data using the BLAST which can be accessed through the NCBI page. (https://www.ncbi.nlm.nih.gov/). Of the 12 samples that were successfully isolated, there were 5 isolates belonging to the genus Streptomyces, namely isolates A, C, E, H, and I as shown in Table 1.

To identify bacterial species, we employed 16S rRNA sequencing followed by BLAST alignment analysis. Each bacterial isolate underwent sequencing to determine its species name based on sequence similarity to known 16S rRNA gene sequences in databases. The resulting alignment metrics, including max score, total score, E-value, query cover, and % identity, were evaluated to assess identification quality. The majority of isolates exhibited high %identity values, typically exceeding 90%, indicating accurate species matching. For instance, isolate A was identified as *Streptomyces* sp. NXHG3 with a 90.59% identity, while isolate C matched to *Streptomyces castaneus* with 91.75%, demonstrating reliable classification. Even isolates with slightly lower % identity values, such as isolate E (*Streptomyces lannensis* at 81.51%), were still confidently identified. To streamline analysis, a "Streptomyces category" was included to indicate species belonging to the *Streptomyces* genus. Several isolates, including A, C, E, H, and I, were categorized as *Streptomyces*, validating the method's effectiveness for identifying species within this genus. Overall, the 16S rRNA sequencing and BLAST analysis approach proved to be a robust and accurate method for bacterial species identification, as evidenced by the high similarity scores and low E-values obtained across all isolates.

The phylogenetic tree in Figure 2 illustrates the evolutionary relationships among sample I and other *Streptomyces* species. The root of the tree represents the common ancestor of all taxa analyzed. The branches on the tree indicate evolutionary divergence over time. Branch lengths generally represent the number of genetic changes that have occurred. The studied sample (marked in blue) branches at a specific point on the tree, indicating that it shares a more recent common ancestor with certain *Streptomyces* species than with others. This branching position suggests a closer relationship between the sample and the

Streptomyces species located on the same branch. Based on this phylogenetic tree, it can be concluded that sample I likely originates from the same evolutionary lineage as the *Streptomyces* species on the closest branch. This indicates a close evolutionary relationship between the sample and these species. It is probable that the sample and these related species share similar phenotypic and genotypic characteristics, such as the ability to produce secondary metabolites or adaptations to specific environments.The phylogeny tree results from 16S rRNA sequencing analysis showed that isolate I was Streptomyces. Based on 16S sequencing data and PCR electrophoresis visualization, breeding was carried out on isolate I. Breeding was carried out on a 1 L Erlenmeyer glass with a liquid volume of 200 mL. Breeding is carried out in an incubator shaker at room temperature for 14 days at a speed of 130 rpm following the previous methods [22][26]-[30].

A list of compounds resulting from HRMS and each mass error is available in Table 2 while their chemical structures are shown in Figure 3. Secondary metabolite profiling from HRMS data concerning the mass error of a maximum of 5 ppm. At a retention time of 8.53 min, the positive ion mode, the compound indicated as *N*-[(2*S*)-1-hydroxy-3-phenylpropan-2-yl]benzamide (NP-001798). It was declared new because it had not been recorded on the mass bank website. According to calculations, this compound has a mass accuracy of 4.0 ppm, so the mass specification is met. The diphenoconazole spectrum, an antifungal commonly used in agriculture, appeared at a retention time of 16.96 min. However, due to the mass accuracy of 5.9 ppm, it is said that the mass

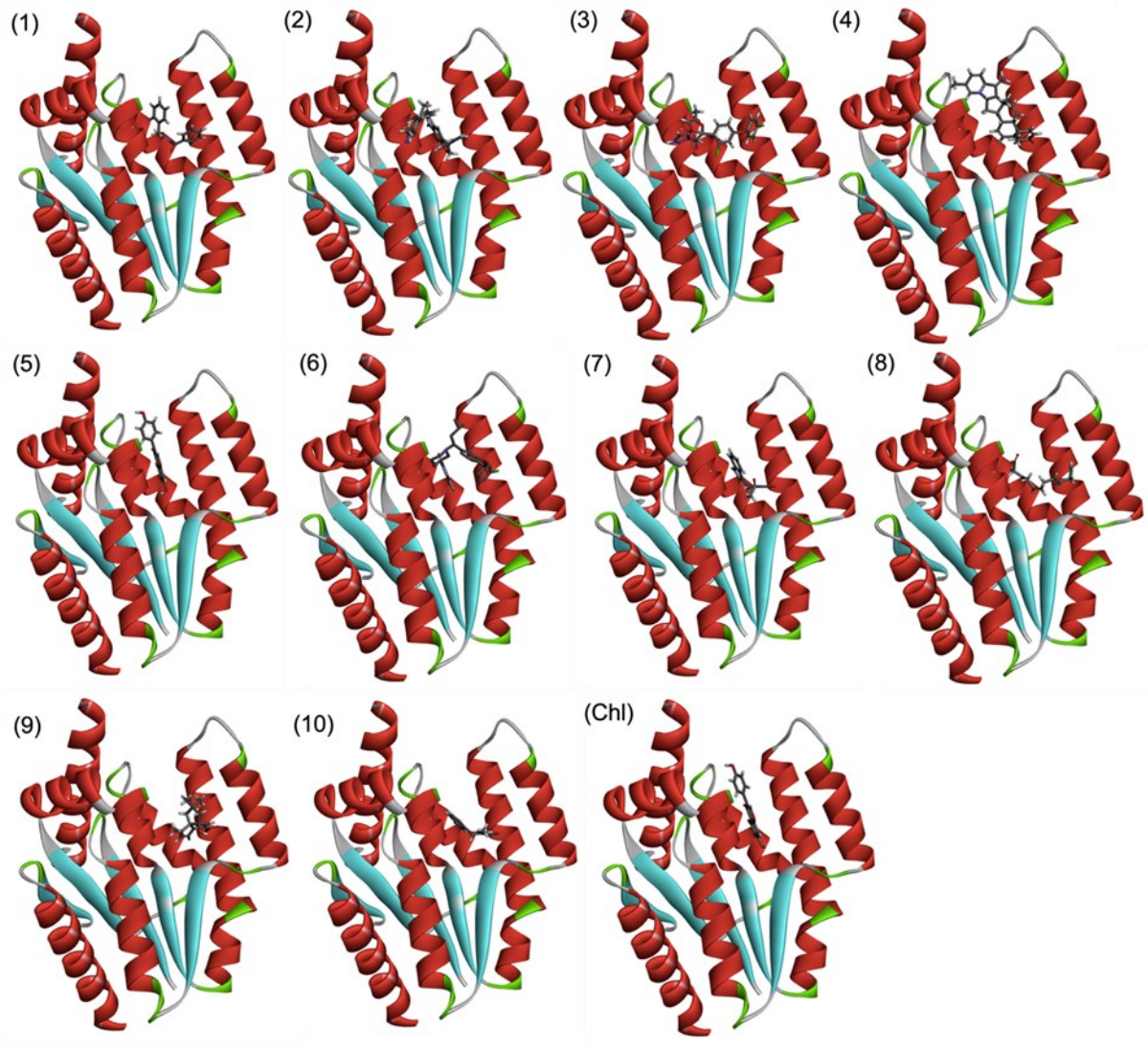

Figure 5. The binding conformation of each compound in the active site of the thymidylate kinase protein.

Table 3. Molecular docking results of the extracted compounds.

Compound	Binding energy (kcal/mol)	Binding constant (μM)	Hydrogen bonding
1	-8.012	1.34	Arg70 and Ser97
2	-7.966	1.45	Lys15 and Arg92
3	-9.658	0.08	-
4	-8.302	0.82	Ser69
5	-7.339	4.17	Gln101
6	-7.242	4.92	Ser69 and Gln101
7	-6.454	18.58	Arg70, Ser97, and Gln101
8	-6.959	7.92	Glu37
9	-7.035	6.97	Glu37 and Ser69
10	-7.686	2.32	Glu37, Gln101, and Arg105
Chloramphenicol	-7.043	6.88	Glu37, Arg70, and Ser97

accuracy is not convincing. At a retention time of 20.09 min, the compound indicated as SB236057A was read with a mass accuracy of 5.5 ppm. This compound, also called serotonin receptor (5HT), is a protein involved in neurological and biological matters [26][27]. One of the compounds that is a sterol, solanidin, is a glycoalkaloid steroid compound found in the vegetable family solaniceae [31]. Daidzein and genistein are flavonoid compounds (isoflavones) derived from soybean plants [32]. These compounds are known to have abilities such as anticancer, antioxidant, anti-inflammatory, and preventing hepatitis C. Both compounds appear in negative ion mode and have an accuracy of less than 2 ppm. The 2-(acetylamino)-3-(1*H*-indole-3-yl)proanoic acid is also read in negative mode and has a mass accuracy of 0.3 ppm. The compound recorded at a retention time of 8.47 min was indicated as (*R*,*Z*)-2-((1-hydroxyethylidene)amino)-3-phenylpropanoic acid, with a calculated mass accuracy of 1.49 ppm. The new compound read at a retention time of 14.28 min was indicated as (*Z*)-3-hydroxydodec-5-enoic acid with a mass accuracy of 1.4 ppm. Meanwhile, at a retention time of 18.59 min, the compound read was indicated as 5a-hydroxy-1,7,7-trimethyl-1,2,3,3a,5a,6,7,8-octahydrocyclopenta[c]pentalene-4-carboxylate. The mass accuracy obtained from the calculation of theoretical mass and measured mass is 0.4 ppm. Difenoconazole compound, which is known as one of the antifungals that are often applied to vegetable plants, is thought to be a residual compound that affects *S. Setonensis* metabolism.

3.2. Antibacterial Activity of The Isolate

The results of the bioactivity test of the extract at 1000 ppm concentration showed the ability to inhibit Gram-positive and Gram-negative bacteria such as *P. acnes, S. aureus,* and *E. coli* with inhibition zones of 14, 14, and 15 mm, respectively (Figure 4). As the positive control, chloramphenicol gave the inhibition zone of 28, 27, and 30 mm, respectively.

3.3. Molecular Docking and Molecular Dynamics Simulations

The molecular docking is done to predict the potential component of the extract that actively inhibits the function of thymidylate kinase protein for a representative study of the antibacterial agents. Thymidylate kinase protein plays a crucial role in the DNA synthesis of *S. aureus*; thus, this protein has been widely used as a potential target for antibacterial agents [33]. At first, each compound in the extract was located in the same Cartesian coordinate as same as the native ligand to evaluate their binding conformation. As the native ligand 4QGH, is inhibitor of TMK enzymes and it could interact with the amino acids Arg48, Arg70, Val51, Leu52, Pro38, Phe66, Gln101, and Ser97 [34][35]. The binding conformation of each compound in the active site of the thymidylate kinase protein is shown in Figure 5 while the formed interactions are

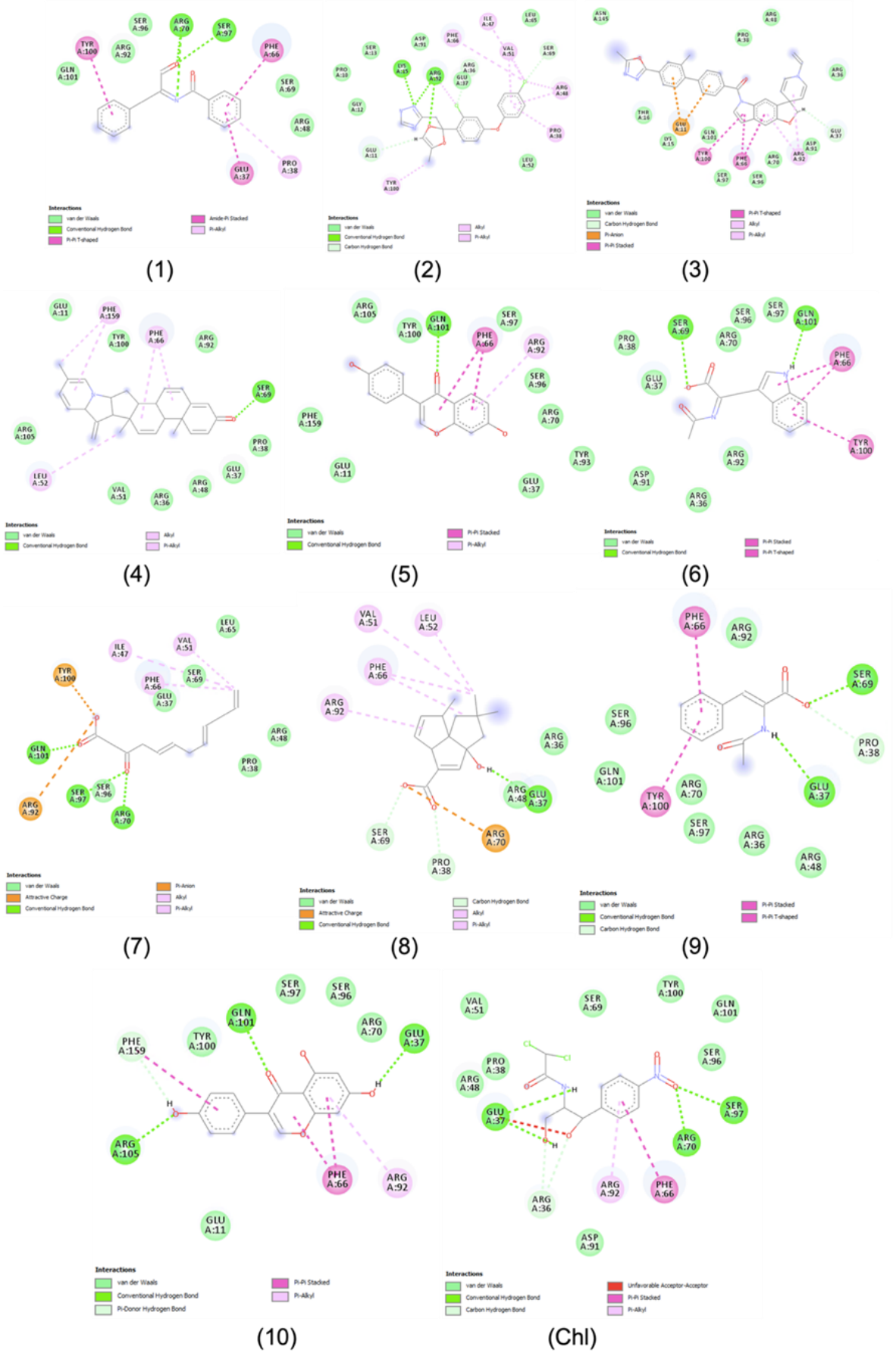

Figure 6. The binding interactions of each compound in the active site of the thymidylate kinase protein.

visualized in Figure 6. Figure 5 shows that all compounds could fit and interact with the active site of the thymidylate kinase protein. From the molecular point of view, each compound in the extract exhibits unique chemical interactions.

The molecular docking data are listed in Table 3. Compound **1** interacted with Arg70 and Ser97 residues via hydrogen bonds; with Glu37, Phe66, and Tyr100 residues via pi-pi T-shaped and amide-pi stacked interactions; with Pro38 residue via pi-alkyl interaction; and with Arg48, Ser69, Arg92, Ser96, and Gln101 residues via van der Waals interaction. These interactions yielded -8.012 kcal/mol binding energy and 1.34 µM binding constant. Compound **2** interacted with Lys15 and Arg92 residues via hydrogen bonds; with Glu11 and Ser69 residues via carbon-hydrogen bonds; with Pro38, Ile47, Arg48, Val51, and Tyr100 residue via alkyl and pi-alkyl interactions; and with Pro10, Gly12, Ser13, Glu37, Leu52, and Leu65 residues via van der Waals interaction. These interactions yielded -7.966 kcal/mol binding energy and 1.45 µM binding constant. Compound **3** interacted with Glu37 residues via carbon-hydrogen bond; with Glu11 via pi-anion interaction; with Phe66 and Tyr100 residues via pi-pi T-shaped and pi-pi stacked interactions; with Arg92 residue via pi-alkyl interaction; and with Lys15, Thr16, Arg36, Pro38, Arg48, Asp70, Asp91, Ser96, Ser97, Gln101, and Asn145 residues via van der Waals

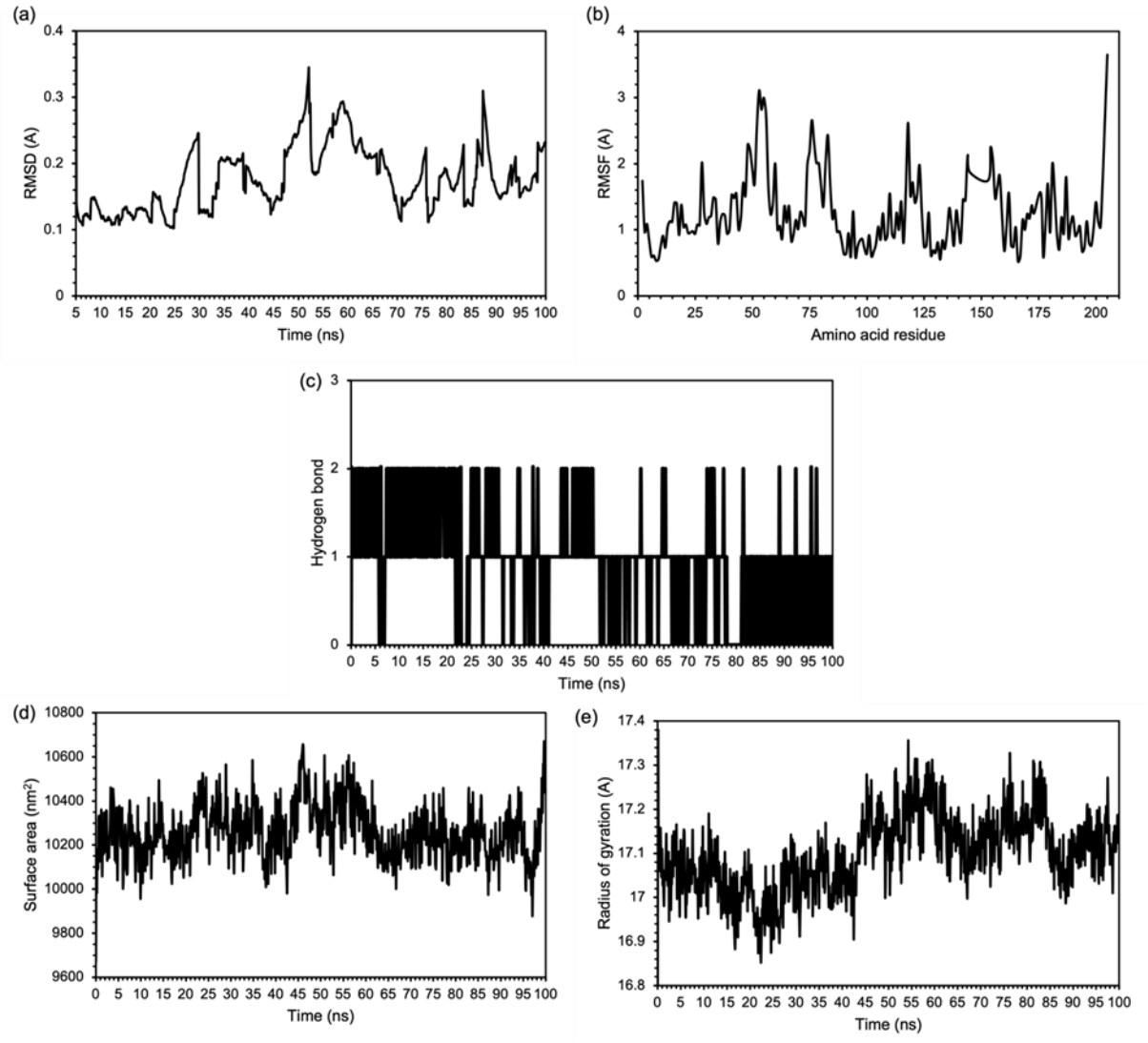

Figure 7. The molecular dynamics simulations results: (a) ΔRMSD, (b) RMSF, (c) carbon-hydrogen bond stability between compound **3** with Glu37 residue in the active site of the thymidylate kinase protein, (d) solvent accessible surface area, and (e) radius of gyration.

interaction. These interactions yielded -9.658 kcal/mol binding energy and 0.08 µM binding constant. Compound **4** interacted with Ser69 residue via hydrogen bond; with Leu52, Phe66, and Phe159 residues via alkyl and pi-alkyl interaction; and with Glu11, Arg36, Glu37, Pro38, Arg48, Val51, Arg92, Tyr100, and Arg105 residues via van der Waals interaction. These interactions yielded -8.302 kcal/mol binding energy and 0.82 µM binding constant. Compound **5** interacted with Gln101 residue via hydrogen bond; with Phe66 residue via pi-pi stacked interaction; with Arg92 residue via pi-alkyl interaction; and with Glu11, Glu37, Arg70, Tyr93, Ser96, Ser97, Tyr100, Arg105, and Phe159 residues via van der Waals interaction. These interactions yielded -7.339 kcal/mol binding energy and 4.17 µM binding constant.

On the other hand, compound **6** interacted with Ser69 and Gln101 residues via hydrogen bonds; with Phe66 and Tyr100 residues via pi-pi T-shaped and pi-pi stacked interactions; and with Arg36, Glu37, Pro38, Arg70, Asp91, Ser96, and Ser97 residues via van der Waals interaction. These interactions yielded -7.242 kcal/mol binding energy and 4.92 µM binding constant. Compound **7** interacted with Arg70, Ser97, and Gln101 residues via hydrogen bonds; with Arg92 and Tyr100 residues via pi-anion and attractive charge interactions; with Ile47, Val51, and Phe66 residues via pi-alkyl interaction; and with Glu37, Pro38, Arg48, Leu65, Ser69, and Ser96 residues via van der Waals interaction. These interactions yielded -6.454 kcal/mol binding energy and 18.58 µM binding constant. Compound **8** interacted with Glu37 residue via hydrogen bond; with Arg70 residues via attractive charge interaction; with Pro38 and Ser69 residues via carbon-hydrogen bond; with Val51, Leu52, Phe66, and Arg92 residues via pi-alkyl interactions; and with Arg36 and Arg48 residues via van der Waals interaction. These interactions yielded -6.959 kcal/mol binding energy and 7.92 µM binding constant. Compound **9** interacted with Glu37 and Ser69 residues via hydrogen bonds; with Pro38 residue via carbon-hydrogen bond; with Pro66 and Tyr100 residues via pi-pi stacked and pi-pi T-shaped interactions; and with Arg36, Arg48, Arg70, Arg92, Ser96, Ser97, and Gln101 residues via van der Waals interaction. These interactions yielded -7.035 kcal/mol binding energy and 6.97 µM binding constant. Compound **10** interacted with Glu37, Gln101, and Arg105 residues via hydrogen bonds; with Phe159 via pi-donor hydrogen bond; with Phe66 and Phe159 residues via pi-pi stacked interactions; with Arg92 residue via pi-alkyl interaction; and with Glu11, Arg70, Ser96, Ser97, and Tyr100 residues via van der Waals interaction. These interactions yielded -7.686 kcal/mol binding energy and 2.32 µM binding constant.

Among the evaluated compounds, compound **3** gave the strongest binding energy (-9.658 kcal/mol), as well as the lowest binding constant (83289.86 pM) due to various chemical interactions, i.e., carbon-hydrogen bond, pi-anion,

Table 4. Drug-likeness analysis of the extracted compounds.

Compound	MW (Da)	Log P	Log S	H donor	H Acceptor	BBB	GI
1	255.31	2.69	1.09×10^{-1}	2	3	Yes	High
2	406.26	2.92	2.10×10^{-3}	1	5	Yes	High
3	534.65	5.05	9.58×10^{-5}	0	6	No	High
4	397.64	4.47	2.87×10^{-4}	1	2	Yes	High
5	254.24	1.77	7.51×10^{-2}	2	4	Yes	High
6	246.26	1.33	2.04	3	3	No	High
7	214.30	2.54	6.94×10^{-1}	2	3	Yes	High
8	250.33	2.25	2.84×10^{-1}	2	3	Yes	High
9	207.23	1.48	1.32	2	4	Yes	High
10	270.24	1.91	5.11×10^{-2}	3	5	No	High
Chloramphenicol	323.13	0.49	1.54	3	5	No	High

pi-pi T-shaped, pi-pi stacked, pi-alkyl, and via van der Waals interaction. As the commercial antibacterial drug, chloramphenicol interacted with Glu37, Arg70, and Ser97 residues via hydrogen bondings; with Arg36 via carbon-hydrogen bond; with Phe66 residue via pi-pi stacked interaction; with Arg92 residue via pi-alkyl interaction; and with Val51, Pro38, Arg48, Asp91, Ser69, Tyr100, Gln101, and Ser96 residues via van der Waals interaction. However, chloramphenicol generated unfavorable acceptor-acceptor interaction with Glu37 residue; thus, weakening its binding energy of -7.043 kcal/mol with a binding constant of 6.88 μM. The binding energy of compound **3** was still stronger than that of chloramphenicol, as well as the binding constant of compound **3** was lower than chloramphenicol, demonstrating the potential application of compound **3** as a new antibacterial agent.

To evaluate the stability of the non-covalent interactions between compound **3** and the amino acid residues of thymidylate kinase protein, the molecular dynamics simulations were investigated. The simulation was performed in a 74.0 × 73.5 × 75.5 Å box containing thymidylate kinase protein with 195 amino acids, as well as 6744 water molecules, 28 sodium ions, and 19 chloride anions to reach a plateau total potential energy of the system at -367000 kJ/mol. The RMSD, RMSF, and carbon-hydrogen bond stability with Glu37 residue results are shown in Figure 7. The RMSD value was expressed as ΔRMSD value in which the first 5 ns was assumed as the equilibration process [23]. The ΔRMSD value of the molecular dynamics simulations was in a range of 0.18 ± 0.06 Å. This value is acceptable as the ΔRMSD value was not higher than 2 Å as the maximum threshold. Furthermore, the RMSF value was also found in 1.27 ± 0.56 Å. This value is also acceptable as the RMSF value was not higher than 3 Å as the maximum threshold. Both ΔRMSD and RMSF values show that the structures of thymidylate kinase protein and compound **3** were stable during the molecular dynamics simulations for 100 ns. Furthermore, the radius of gyration and solvent accessible surface area data for compound **3** were found to be 17.10 ± 0.09 Å and 10259 ± 124.91 nm^2, respectively, showing the compactness of the complex structure. These results were confirmed by the observation of the carbon-hydrogen bond with Glu37 until the end of the simulation time. From these results, it can be concluded that compound **3** is recommended for antibacterial agent application due to its unique and stable chemical interactions with the thymidylate kinase protein of *S. aureus*.

Further drug-likeness analysis was also performed to evaluate the pharmacokinetic parameters of the extracted compounds as listed in Table 4. This analysis pays attention to Lipinsky's rule, which is often called the Rule of Five, i.e., molecular mass less than 500 Da, log P less than 4.15, hydrogen bond donor less than 5, and the number of hydrogen bond acceptors less than 10. Compounds that meet this rule are stated to have drug-likeness properties. All extracted compounds, only compound **6** that has Lipinsky's rule violation, as log P > 4.5. However, the results of the drug-likeness shows that all compounds have high probability of being absorbed passively by the digestive tract. Further blood-brain barriers (BBB) permeability parameter stated that compounds **1**, **2**, **4**, **5**, **7**, **8**, and **9** could cross BBB while all compounds exhibited high gastrointestinal (GI) absorption. It means all compounds meet the Lipinsky regulation, so they are easy to adsorb, and distributed through the bloodstream, although cell membranes in different tissues have different permeability to drugs. Blood flow to the tissue will also affect the rate of drug distribution. Tissues with high perfusion (e.g. heart, kidneys) will receive the drug faster. Compound **6**, has the most permeability skin so that it penetrates the skin most easily The metabolism of compounds in the liver will be affected by the pharmacokinetic parameters of CYP1A2 in compounds **1**, **2**, and **10** showing that they inhibit phase 1 metabolism in the liver.

4. CONCLUSIONS

The Streptomyces has been successfully isolated from mangrove sediments from the Baros coast of Yogyakarta which has a resemblance level of 95.44% to Streptomyces sp. The compound profile produced by Streptomyces isolates I is in the form of benzimidic acid, diphenoconazole, SB, solanines (sterols), daidzein (flavonoids), propanoic acid, NP (carboxylic acid), NP (propanoic acid), and genistein (flavonoid). *In vitro* testing showed that

the extract was able to inhibit *P. acne*, *S. aureus*, and *E. coli* bacteria. Further *in silico* tests with molecular docking showed that compound **3** was able to bind well to the thymidylate kinase protein, so it is suspected that this compound has the ability to be a promising antibacterial agent, as supported by the molecular dynamics simulations. According to drug-likeness analysis, the chemical compound exhibits good bioavailability and pharmacokinetic properties. This finding contributes to the knowledge of microbial biodiversity in mangrove sediments.

AUTHOR INFORMATION

Corresponding Author

Winarto Haryadi — Department of Chemistry, Universitas Gadjah Mada, Yogyakarta-55281 (Indonesia);

orcid.org/0000-0001-7970-1528

Email: wnrt_haryadi@ugm.ac.id

Authors

Dwi Marwati Juli Siswanti — Department of Chemistry, Universitas Gadjah Mada, Yogyakarta-55281 (Indonesia); Department of Chemistry, Universitas Mathla'ul Anwar, Pandeglang-42273 (Indonesia);

orcid.org/0009-0006-4741-3031

Kasta Gurning — Department of Chemistry, Universitas Gadjah Mada, Yogyakarta-55281 (Indonesia); Department of Pharmacy, Sekolah Tinggi Ilmu Kesehatan Senior Medan, Medan-20141 (Indonesia);

orcid.org/0000-0002-0676-0030

Chairil Anwar — Department of Chemistry, Universitas Gadjah Mada, Yogyakarta-55281 (Indonesia);

orcid.org/0000-0002-5189-1531

Respati Tri Swasono — Department of Chemistry, Universitas Gadjah Mada, Yogyakarta-55281 (Indonesia);

orcid.org/0000-0003-0762-6930

Author Contributions

Conceptualization, C. A., W. H., and R. T. S.; Methodology, W. H., and R. T. S.; Software, W. H.; Formal Analysis, D. M. J. S.; Investigation, D. M. J. S.; Resources, C. A. and W. H.; Data Curation, D. M. J. S.; Writing – Original Draft Preparation, D. M. J. S. and K. G.; Writing – Review & Editing, D. M. J. S., K. G., and W. H.; Funding Acquisition, C. A.

Conflicts of Interest

The authors declare no conflict of interest.

ACKNOWLEDGEMENT

This study was supported financially by Universitas Gadjah Mada under the scheme of PDD research grant (Contract number: 2257/UN1/DITLIT/DIT-LIT/PT/2021).

REFERENCES

[1] M. Liu, E. M. El-Hossary, T. A. Oelschlaeger, M. S. Donia, R. J. Quinn, and U. R. Abdelmohsen. (2019). "Potential of marine natural products against drug-resistant bacterial infections". *The Lancet Infectious Diseases*. **19** (7): e237-e245. 10.1016/S1473-3099(18)30711-4.

[2] R. Kusdarwati, I. Pratiwi, and W. Tjahjaningsih. (2013). "Eksplorasi Bakteri Kandidat Probiotik di Lumpur Hutan Mangrove Wonorejo
<i>[Exploration The Candidate Probiotic Bacteria In Mangrove Mud Wonorejo]<I>". *Jurnal Ilmiah Perikanan dan Kelautan*. **5** (2): 187-192. 10.20473/jipk.v5i2.11407.

[3] G. A. Quinn, A. M. Banat, A. M. Abdelhameed, and I. M. Banat. (2020). "Streptomyces from traditional medicine: sources of new innovations in antibiotic discovery". *Journal of Medical Microbiology*. **69** (8): 1040-1048. 10.1099/jmm.0.001232.

[4] A. Sarmiento-Vizcaino, J. Martin, F. J. Ortiz-Lopez, F. Reyes, L. A. Garcia, and G. Blanco. (2022). "Natural products, including a new caboxamycin, from Streptomyces and other Actinobacteria isolated in Spain from storm clouds transported by Northern winds of Arctic origin". *Frontiers in Chemistry*. **10** : 948795. 10.3389/fchem.2022.948795.

[5] K. Yeshi, D. Crayn, E. Ritmejeryte, and P. Wangchuk. (2022). "Plant Secondary

Metabolites Produced in Response to Abiotic Stresses Has Potential Application in Pharmaceutical Product Development". *Molecules.* **27** (1). 10.3390/molecules27010313.

[6] B. R. Bhattarai, K. Khadayat, N. Aryal, B. Aryal, U. Lamichhane, K. Bhattarai, N. Rana, B. P. Regmi, A. Adhikari, S. Thapa, and N. Parajuli. (2022). "Untargeted Metabolomics of Streptomyces Species Isolated from Soils of Nepal". *Processes.* **10** (6). 10.3390/pr10061173.

[7] Z. Yu, C. Han, B. Yu, J. Zhao, Y. Yan, S. Huang, C. Liu, and W. Xiang. (2020). "Taxonomic Characterization, and Secondary Metabolite Analysis of Streptomyces triticiradicis sp. nov.: A Novel Actinomycete with Antifungal Activity". *Microorganisms.* **8** (1). 10.3390/microorganisms8010077.

[8] M. L. Hui, L. T. Tan, V. Letchumanan, Y. W. He, C. M. Fang, K. G. Chan, J. W. Law, and L. H. Lee. (2021). "The Extremophilic Actinobacteria: From Microbes to Medicine". *Antibiotics (Basel).* **10** (6). 10.3390/antibiotics10060682.

[9] K. Alam, A. Mazumder, S. Sikdar, Y. M. Zhao, J. Hao, C. Song, Y. Wang, R. Sarkar, S. Islam, Y. Zhang, and A. Li. (2022). "Streptomyces: The biofactory of secondary metabolites". *Frontiers in Microbiology.* **13** : 968053. 10.3389/fmicb.2022.968053.

[10] S. Ratnakomala, P. Lisdiyanti, N. R. Prayitno, E. Triana, Y. Lestari, R. D. Hastuti, Y. Widyastuti, M. Otoguro, K. Ando, and E. Sukara. (2016). "Diversity of Actinomycetes from Eka Karya Botanical Garden, Bali". *Biotropia.* **23** (1): 42-51. 10.11598/btb.2016.23.1.504.

[11] I. Handayani, S. Ratnakomala, P. Lisdiyanti, Fahrurrozi, M. Alanjary, W. Wohlleben, and Y. Mast. (2018). "Complete Genome Sequence of Streptomyces sp. Strain BSE7F, a Bali Mangrove Sediment Actinobacterium with Antimicrobial Activities". *Genome Announcements.* **6** (26). 10.1128/genomeA.00618-18.

[12] J. W. Law, K. G. Chan, Y. W. He, T. M. Khan, N. S. Ab Mutalib, B. H. Goh, and L. H. Lee. (2019). "Diversity of Streptomyces spp. from mangrove forest of Sarawak (Malaysia) and screening of their antioxidant and cytotoxic activities". *Scientific Reports.* **9** (1): 15262. 10.1038/s41598-019-51622-x.

[13] C. Wang, Y. Lu, and S. Cao. (2020). "Antimicrobial compounds from marine actinomycetes". *Archives of Pharmacal Research.* **43** (7): 677-704. 10.1007/s12272-020-01251-0.

[14] E. Cho, O. S. Kwon, B. Chung, J. Lee, J. Sun, J. Shin, and K. B. Oh. (2020). "Antibacterial Activity of Chromomycins from a Marine-Derived Streptomyces microflavus". *Marine Drugs.* **18** (10). 10.3390/md18100522.

[15] L. Donald, A. Pipite, R. Subramani, J. Owen, R. A. Keyzers, and T. Taufa. (2022). "Streptomyces: Still the Biggest Producer of New Natural Secondary Metabolites, a Current Perspective". *Microbiology Research.* **13** (3): 418-465. 10.3390/microbiolres13030031.

[16] N. M. Fahmy. (2020). "Isolation and characterization of Streptomyces sp. NMF76 with potential antimicrobial activity from mangrove sediment, Red Sea, Egypt". *Egyptian Journal of Aquatic Biology and Fisheries.* **24** (6): 479-495. 10.21608/ejabf.2020.117578.

[17] K. Gurning, S. Suratno, E. Astuti, and W. Haryadi. (2024). "Untargeted LC/HRMS Metabolomics Analysis and Anticancer Activity Assay on MCF-7 and A549 Cells from Coleus amboinicus Lour Leaf Extract". *Iranian Journal of Pharmaceutical Research.* **23** (1): e143494. 10.5812/ijpr-143494.

[18] S. Rampone, C. Pagliarulo, C. Marena, A. Orsillo, M. Iannaccone, C. Trionfo, D. Sateriale, and M. Paolucci. (2021). "In silico analysis of the antimicrobial activity of phytochemicals: towards a technological breakthrough". *Computer Methods and Programs in Biomedicine.* **200** : 105820. 10.1016/j.cmpb.2020.105820.

[19] W. Haryadi and H. D. Pranowo. (2023). "Molecular docking and dynamics analysis of

halogenated imidazole chalcone as anticancer compounds". *Pharmacia.* **70** (2): 323-329. 10.3897/pharmacia.70.e101989.

[20] W. Haryadi, K. Gurning, and E. Astuti. (2024). "Molecular target identification of two Coleus amboinicus leaf isolates toward lung cancer using a bioinformatic approach and molecular docking-based assessment". *Journal of Applied Pharmaceutical Science.* 10.7324/japs.2024.164753.

[21] Y. S. Kurniawan, K. Gurning, I. Iksen, and A. Bikharudin. (2024). "Fight for Cancer Diseases using Natural Compounds and Their Semisynthetic Derivatives". *Bioactivities.* 10.47352/bioactivities.2963-654X.221.

[22] E. Damayanti, P. Lisdiyanti, A. Sundowo, S. Ratnakomala, A. Dinoto, J. Widada, and M. Mustofa. (2021). "Antiplasmodial activity, biosynthetic gene clusters diversity, and secondary metabolite constituent of selected Indonesian Streptomyces". *Biodiversitas Journal of Biological Diversity.* **22** (6). 10.13057/biodiv/d220657.

[23] Y. S. Kurniawan, E. Yudha, G. Nugraha, N. Fatmasari, H. D. Pranowo, J. Jumina, and E. N. Sholikhah. (2024). "Molecular Docking and Molecular Dynamic Investigations of Xanthone-Chalcone Derivatives against Epidermal Growth Factor Receptor for Preliminary Discovery of Novel Anticancer Agent". *Indonesian Journal of Chemistry.* **24** (1). 10.22146/ijc.88449.

[24] M. Fouillaud and L. Dufosse. (2022). "Microbial Secondary Metabolism and Biotechnology". *Microorganisms.* **10** (1). 10.3390/microorganisms10010123.

[25] J. S. Johnson, D. J. Spakowicz, B. Y. Hong, L. M. Petersen, P. Demkowicz, L. Chen, S. R. Leopold, B. M. Hanson, H. O. Agresta, M. Gerstein, E. Sodergren, and G. M. Weinstock. (2019). "Evaluation of 16S rRNA gene sequencing for species and strain-level microbiome analysis". *Nature Communications.* **10** (1): 5029. 10.1038/s41467-019-13036-1.

[26] R. Armin, S. Zuhlke, G. Grunewaldt-Stocker, F. Mahnkopp-Dirks, and S. Kusari. (2021). "Production of Siderophores by an Apple Root-Associated Streptomyces ciscaucasicus Strain GS2 Using Chemical and Biological OSMAC Approaches". *Molecules.* **26** (12). 10.3390/molecules26123517.

[27] R. Armin, S. Zühlke, F. Mahnkopp-Dirks, T. Winkelmann, and S. Kusari. (2021). "Evaluation of Apple Root-Associated Endophytic Streptomyces pulveraceus Strain ES16 by an OSMAC-Assisted Metabolomics Approach". *Frontiers in Sustainable Food Systems.* **5**. 10.3389/fsufs.2021.643225.

[28] N. A. Al-Dhabi, G. A. Esmail, A. M. Ghilan, M. V. Arasu, V. Duraipandiyan, and K. Ponmurugan. (2020). "Chemical constituents of Streptomyces sp. strain Al-Dhabi-97 isolated from the marine region of Saudi Arabia with antibacterial and anticancer properties". *Journal of Infection and Public Health.* **13** (2): 235-243. 10.1016/j.jiph.2019.09.004.

[29] L. Sarao, S. Kaur, T. Malik, and A. Singh. (2022). In: "Nutraceuticals and Health Care". 331-341. 10.1016/b978-0-323-89779-2.00016-8.

[30] P. Chandra, R. K. Sharma, and D. S. Arora. (2020). "Antioxidant compounds from microbial sources: A review". *Food Research International.* **129** : 108849. 10.1016/j.foodres.2019.108849.

[31] M. S. Almuhayawi, M. S. M. Mohamed, M. Abdel-Mawgoud, S. Selim, S. K. Al Jaouni, and H. AbdElgawad. (2021). "Bioactive Potential of Several Actinobacteria Isolated from Microbiologically Barely Explored Desert Habitat, Saudi Arabia". *Biology (Basel).* **10** (3). 10.3390/biology10030235.

[32] S. Satarker, P. C. Gurram, A. Nassar, S. Manandhar, R. Vibhavari, D. L. Yarlagadda, J. Mudgal, S. Lewis, D. Arora, and M. Nampoothiri. (2024). "Evaluating the Role of N-Acetyl-L-Tryptophan in the Abeta 1-42-Induced Neuroinflammation and Cognitive Decline in Alzheimer's Disease". *Molecular Neurobiology.* **61** (7): 4421-4440. 10.1007/s12035-023-03844-4.

[33] B. Qureshi, R. Khalil, M. Saeed, E. A. M. Nur, S. Ahmed, and Z. Ul-Haq. (2022).

"Structure-Based Discovery of Potent Staphylococcus aureus Thymidylate Kinase Inhibitors by Virtual Screening". *Medicinal Chemistry.* **19** (1): 75-90. 10.2174/1573406418666220407092638.

[34] K. Jayanthi and M. A. Azam. (2023). "Thymidylate Kinase Inhibitors as Antibacterial Agents: A Review". *Applied Biochemistry and Microbiology.* **59** (3): 250-266. 10.1134/s0003683823030092.

[35] S. P. Kawatkar, T. A. Keating, N. B. Olivier, J. N. Breen, O. M. Green, S. Y. Guler, M. F. Hentemann, J. T. Loch, A. R. McKenzie, J. V. Newman, L. G. Otterson, and G. Martinez -Botella. (2014). "Antibacterial inhibitors of Gram-positive thymidylate kinase: structure-activity relationships and chiral preference of a new hydrophobic binding region". *Journal of Medicinal Chemistry.* **57** (11): 4584-97. 10.1021/jm500463c.