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Isolation of Secondary Metabolite Compounds from the Soft Coral *Isis sp*., Antimicrobial Testing and Molecular Docking Studies

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Abstract: Soft coral *Isis* sp. is a type of soft coral that contains many chemical compounds with various benefits that have not been widely studied. This study aims to identify and isolate secondary metabolite compounds contained in the ethyl acetate extract fraction of *Isis* sp. soft coral, determine of antimicrobial activity, and molecular docking study. Compound separation was carried out by maceration followed by fractionation using Liquid Vacuum Chromatography (LVC). The fractions obtained were identified by Liquid Chromatography Mass Spectroscopy/Mass Spectroscopy (LC-MS/MS) (LC-MS/MS) technique. Four compounds were successfully identified through LC-MS/MS analysis namely Deoxyadenosine ($C_{10}H_{13}N_5O_3$), Spinasterol ($C_{29}H_{48}O$), Pseudoionone ($C_{11}H_{16}O$) and Isosalsoline ($C_{11}H_{15}NO_2$). *Antimicrobial* activity test was conducted against *Staphylococcus aureus*, and *Escherichia coli*. The results of the antimicrobial activity test showed strong activity against *S.aureus* bacteria and *E. coli* bacteria with the same MIC value of 2 µg/mL. Based on molecular docking analysis can be predicted that compounds isolated from soft coral *Isis sp.*, namely pseudoinone compounds have a role as anti-bacterial *S. aureus* (PDB: 6KVS). This compound also shows a strong ability as an anti-gram positive bacteria than gram negative. Isosalsoline compounds have a role as anti-bacterial *E. coli* (PDB: 5BNM). This compound also shows a strong ability as an anti-gram negative bacteria rather than gram positive.

Keywords: Antimicrobial; *Isis* sp; Soft coral; Maceration; MIC; Molecular docking.

Introduction

Indonesia is strategically located between the continents of Australia and Asia, bordered by the Pacific Ocean and the Indian Ocean, which results in a maritime area significantly larger than its landmass. This extensive marine territory endows Indonesia with substantial potential in the marine sector. The nation's marine biodiversity is exceptionally rich. presenting considerable economic opportunities. Indonesia's maritime economic potential encompasses renewable natural resources including fisheries, mangrove forests, seaweed, biotechnology products, and coral reefs (Rohmat et al., 2023)

Coral reefs represent a unique ecosystem that provides various ecological, economic, tourism, chemical, and biological functions. Generally, coral reefs are categorized into two types: *hard* corals and *soft* corals.

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Soft corals are among the most productive marine organisms in terms of secondary metabolites. They possess soft and flexible bodies with stalks that attach to hard substrates (Asih et al., 2021; Koroy et al., 2020).

Soft corals are abundant in bioactive compounds, including terpenoids, steroids, alkaloids, flavonoids, phenols, and saponins. The soft coral genus *Isis* sp. serves as a notable example found in tropical Indo-Pacific waters (Eissa et al., 2024). This genus is recognized for its beneficial applications in the pharmaceutical industry due to its chemical compounds, which can serve as antibiotic and antiviral agents. In medicine, *Isis* sp. has demonstrated the ability to slow down and prevent viral proliferation, as well as inhibit the spread of cancer cells. Additionally, *Isis* sp. exhibits antibacterial, antiviral, and anticancer properties (Prasetia et al., 2022). The bioactive components of *Isis* sp. extract include various alkaloids, phenolic compounds, steroids, and flavonoids. Flavonoids exhibit antibacterial properties by compromising bacterial cell walls and membranes, while steroid bioactive components have been reported to inhibit the growth of both Gram-positive and Gram-negative bacteria (Muliadin et al., 2022).

Infectious diseases persist as significant health challenges, constituting a leading cause of elevated morbidity and mortality, particularly in developing countries. Pathogenic bacteria, such as *Staphylococcus aureus* (a gram-positive organism) and *Escherichia coli* (a gram-negative organism), are commonly found in community environments. To mitigate the proliferation of these pathogenic bacteria, it is essential to employ antibacterial compounds (Magani et al., 2020). Antibacterial compounds refer to substances or agents that possess the ability to inhibit bacterial growth (bacteriostatic or fungistatic) or to eradicate bacteria or molds (bactericidal or fungicidal) (Dey et al., 2025).

Resistance refers to the capacity of bacteria or other microorganisms to endure and proliferate in response to antibiotic doses that were previously deemed effective (Irsyaadyah, 2019). Antimicrobial resistance poses a significant challenge as it diminishes treatment efficacy, amplifies the transmission of infections, and escalates healthcare costs. The rising incidence of resistance cases is partially attributed to the lack of new antibiotic discoveries (Handayani et al., 2018).

Elevated levels of antimicrobial resistance can also be linked to the misuse of antibiotics. Thus, the discovery of new antimicrobial compounds is of paramount importance. One method employed to identify antimicrobial compounds is the *broth microdilution* technique. This microdilution method represents an advancement of the liquid dilution method, utilizing minimal quantities of media, bacteria, and test compounds in conjunction with a *96-well* microplate. The primary advantage of this method is its efficiency, as it requires only small volumes of media and samples (Weng et al., 2025)

The objective of this study was to isolate secondary metabolite compounds from the ethyl acetate fraction of *Isis sp.* soft coral and determine their antimicrobial activity, and analyze the interaction of these metabolite compounds using molecular docking.

Method

Times and Place of Research

This research was conducted in 2023, took place at the Pharmaceutical Natural Materials Research Laboratory, Faculty of Pharmacy, Halu Oleo University, Kendari, Southeast Sulawesi for isolation of *Isis* sp. soft coral samples, as well as microbial activity tests. Identification of secondary metabolite compounds using LC-MS/MS was carried out at the National Research and Innovation Agency (BRIN), Serpong.

Tools and Materials

The tools employed in this investigation comprises: a liquid vacuum chromatography (LVC) apparatus, a rotary vacuum evaporator (Buchi®), an oven (Stuart®), an analytical balance (Explorer Ohaus®); Si-Gel F254 TLC plates (Merck®), a dropper, a micropipette (Eppendorf®), vial bottles, ordinary filter paper and Whatman No.1 filter paper, a knife, an Erlenmeyer flask (Pyrex®), a UV lamp (UVG-58), a chamber, a cutter, a spatula, tweezers, a capillary tube; beakers (Pyrex®), measuring cups (Pyrex®), petri dishes, a spatula, an autoclave (Daihan Labtech®), micro pipette tips, a Multiskan FC microplate reader (Thermo Scientific®), and F-bottom 96-well microplates (Greiner Bio-One®), AutoDockTools 1.5.7, Discovery Studio Visualizer, MarvinSketch version 5.2.5.1. The materials utilized in this study include: *Isis* sp. soft coral; methanol (technical grade); acetone (technical grade); ethyl acetate (technical grade); n-hexane(technical grade); silica gel 60 GF254 p.a (Merck®); silica gel 60 G (Merck®); distilled water; cerium sulfate ($CeSO_4$) (Merck®); dimethyl sulfoxide (DMSO); sulfuric acid; barium chloride; *Staphylococcus aureus* ATCC 25923; *Escherichia coli* ATCC 35218; Mueller Hinton Broth (MHB), Mueller Hinton Agar (MHA), Potato Dextrose Agar (PDA), Sabouraud Dextrose Broth (SDB), chloramphenicol, NaCl 0.9%, and chloramphenicol.

Extraction Process

Isis sp. samples, which have been sectioned, are subjected to maceration for three cycles of 24 hours each, utilizing 5 liters of solvent in a closed container, with solvent replacement occurring through the use of ethyl acetate. Ethyl acetate is employed due to its insolubility

in water, making it an appropriate solvent for *Isis* sp. samples that possess a high water content. Subsequently, the mixture is filtered to isolate the filtrate. The obtained filtrate is then concentrated using a rotary vacuum evaporator at 40°C to yield a concentrated extract. The extract is subsequently weighed to determine the sample weight.

Compound Fractionation

Ethyl acetate extract of *Isis* sp. soft coral was fractionated by means of liquid vacuum chromatography (LVC). The stationary phase was silica gel GF254 and the mobile phase used was n-hexane: ethyl acetate in the ratio of (9:1. 8:2. 7:3. 5:5. 2:8. 1:9. 0:10)% and 100% methanol. Eluents from LVC were collected in dark bottles with a volume of 100 mL each.

Antimicrobial Activity Test

Sterilization of Tools and Materials

All tools utilized must be sterilized in advance in accordance with the specific requirements of each tool and material involved. For high heat-resistant glassware, dry sterilization was conducted using an oven set to 160°C for a duration of two hours, with the glassware previously wrapped in aluminum foil. Media and distilled water were sterilized through wet sterilization via autoclaving at 121°C, under a pressure of 15-17.5 psi *(pounds per square* inch), for a period of 15 minutes. Equipment such as hoses, needles, and spatulas can be sterilized with a Bunsen burner by heating the tips of the instruments over the flame until they glow. The combustion process creates airflow, as oxygen is consumed from below, which is anticipated to incinerate contaminants within the airflow pattern.

Media Preparation and McFarland Standard

A total of 8.5 grams of MHA and 5.25 grams of MHB were each dissolved in 250 mL of distilled water in an Erlenmeyer flask, subsequently heated to boiling, and sterilized by autoclaving at 121°C for 15 minutes under a pressure of 15 psi. Additionally, 9.75 grams of PDA and 16.25 grams of SDB were each dissolved in 250 mL of distilled water in an Erlenmeyer flask, heated to boiling, and then sterilized by autoclaving at 121°C for 15 minutes under a pressure of 15 psi. The preparation of the McFarland standard involved the addition of 0.05 mL of 1% barium chloride ($BaCl_2$) in distilled water to 9.95 mL of 1% sulfuric acid (H_2SO_4), with the mixture being stirred until homogeneous. This preparation was then stored in a location protected from direct sunlight.

Inoculum Preparation of Staphylococcus aureus and Eschericia coli Bacteria

Each test bacterium was initially inoculated in Mueller-Hinton agar (MHA) growth medium and incubated at 37°C for 24 hours. Subsequently, each bacterium was suspended in sterile physiological saline (NaCl) solution. The turbidity of the bacterial suspensions was measured according to the 0.5 McFarland standard using a UV-Vis spectrophotometer, with physiological saline solution utilized as a blank.

Antimicrobial Test with Broth Microdillution Method

A total of 100 µL of media was aliquoted into each well of the 96-well microplate. The first column was filled with 100 µL of isolate with a concentration of 512 µg/mL. Subsequently, 100 µL of the mixture from the first column was transferred to the second column, and this process continued sequentially until the eighth column, where the isolate concentration in the last column was halved compared to the previous column. Additionally, 100 µL of microbial suspension was added to each well to ensure that the microbial concentration matched the 0.5 McFarland standard. In the tenth column, 100 µL of DMSO was introduced as a negative control. The eleventh column contained media with microbial suspension only, serving as a growth control, while the twelfth column consisted solely of 200 µL of media as a media control to assess sterility. The mixtures of microbial suspensions and isolates in the media were incubated for 16-20 hours at 37°C for bacteria and for 20 hours at 35°C for fungi. The *Minimum Inhibitory Concentration* (MIC) value was determined by assessing the turbidity of the mixtures at each concentration using spectrophotometric techniques.

MIC determination

Antibacterial and antifungal activity testing was conducted utilizing the Broth Microdilution method to ascertain the *Minimum Inhibitory Concentration* (MIC) value. The MIC is defined as the lowest concentration of the sample that inhibits microbial growth, which can be evaluated by observing the activity of the sample at its minimal dilution within a microplate containing growth media and microbial inoculum. The determination of the MIC value can be assessed visually through the formation of clear media, or by employing spectrophotometric methods, with absorbance values approaching zero indicating no turbidity, and higher values indicating the presence of turbidity.

Determination of Compound Structure by LC-MS/MS

Each extract was identified for chemical content using LC-MS/MS analysis, Xevo G2 XS QTOF (Waters Corporation, Milford, USA) equipped with an electrospray ionization (ESI) source.

ADME Prediction

Prior to molecular docking test, the four compounds that have been isolated are tested for

absorption, distribution, metabolism and excretion (ADME). This is done to ensure that these compounds can be active as antimicrobials.

Molecular Docking Studies

In silico molecular docking is designed to investigate ligand-biomolecular interactions and the potential binding geometries of a proposed ligand with a target protein. To identify optimal targets in microbial cells, three-dimensional (3D) crystal structures of target proteins were utilized, including: (1) DNA gyrase from *E. coli* (PDB: 1S14), (2) DNA gyrase from *P. aeruginosa* (PDB: 6M1S), (3) RNA polymerase from *B. subtilis* (PDB: 4NC7), (4) DHFR from *C. albicans* (PDB: 1AOE), (5) FabH from *E. coli* (PDB: 5BNM), and (6) FabH from *S. aureus* (PDB: 6KVS). These structures were retrieved from www.rcsb.org. Docking simulations were conducted using the AutoDockTools 1.5.7 software with subsequent analysis of molecular interactions performed using Discovery Studio Visualizer (DSV) 2025.

The two-dimensional (2D) structures of the ligands were constructed using MarvinSketch version 5.2.5.1. These structures were then converted to three-dimensional forms to calculate the energy of optimal geometry (E_{total}) using the MMFF94 method. and subsequently saved in *.mol2 format. To prepare the proteins for docking. the 3D structures of the target proteins in PDB format were imported into the AutoDockTools 1.5.7 workspace. The binding site containing the co-crystallized ligand was identified as a cavity. The 3D structure of the test ligand was aligned with the co-crystallized ligand. which served as the reference ligand. Molecular docking was performed following the AutoDockTools 1.5.7 protocol using default parameters. Six reference ligands were employed in separate docking validations corresponding to each protein target.

Result and Discussion

Extraction result

The maceration process was conducted over three 24-hour periods, with intermittent stirring employed to enhance solvent interaction with *Isis* sp., using ethyl acetate as the solvent. Ethyl acetate is recognized for its universal ability to attract both polar and non-polar organic compounds (Situmeang et al., 2025). This solvent is classified as a semi-polar compound, characterized by the presence of an ethoxy group capable of forming hydrogen bonds. Following the maceration, the resultant mixture was filtered, and the solvent was removed via rotary vacuum evaporation. The filtration process allowed for the separation of the filtrate from the residue. Subsequently, the filtrate was concentrated, yielding a thick extract weighing 11 grams from an initial mass of 1 kg of simplicial. Consequently, the percent yield of the extract obtained was calculated to be 1.1%.

Compounds Fractionation

The separation process of secondary metabolite compounds from *Isis* sp. Samples involves the application of Thin Layer Chromatography (TLC) and Liquid Vacuum Chromatography (LVC). Separation occurs as the sample is distributed between two phases: a stationary phase, typically consisting of silica gel, and a mobile phase, which is a mixture of two or more solvents (eluent). The stationary phase functions to adsorb secondary metabolite compounds, while the mobile phase facilitates the movement and elution of these compounds. Prior to separation via the LVC method, the ethyl acetate fraction is subjected to optimization through the TLC method. This optimization process aims to identify the solvent system that effectively separates the compounds present in the ethyl acetate fraction.

Based on the literature, the combination of hexane and ethyl acetate solvents is a universal eluent system recommended in chromatography whose stationary phase uses silica gel. Due to both solvents have low polarity are easy to evaporate and easy to regulate their polarity. Hexane (H) and ethyl acetate (EtOAc) solvents were varied in volume ratio as follows: 9:1 (A). 8:2 (B). 7:3 (C). 6:4 (D). 5:5 (E). 4:6 (F). 3:7 (G). 2:8 (H). 1:9 (I). The results of the chromatogram of the TLC stain profile show that the H: EtOAc eluent can be used in the separation of compounds using LVC because both solvents have the ability to separate the compounds contained in the ethyl acetate fraction.

Based on the chromatogram, the comparison of solvents has a good separation pattern. So, it can be used as an eluent in the separation using the LVC method. Eluents were made in the amount of 150 mL with various comparisons namely A (two times), B (fourth times), C (two times), E (two times), H (two times), I (two times), EtOAc (one times), and finally washed with methanol (MeOH) until finished. Each eluent comparison is made repeatedly so that the separation of compounds is getting better

A total of 100 mL of solvent system was eluted in LVC using silica gel GF254 stationary phase which already contained 22 g of silica gel 60 impregnated sample. The solvent system passed through the column with the help of vacuum and caused the distribution of compounds contained in the ethyl acetate fraction based on their ability to interact with silica gel in the column and the compounds attracted by the solvent system were collected as fractions. The fractions obtained were then photographed on a TLC plate to see the compound

separation pattern based on the uniformity of the Rf value of the spot stain on the chromatogram. The TLC stain profile of the LVC results can be seen in Figure 1.

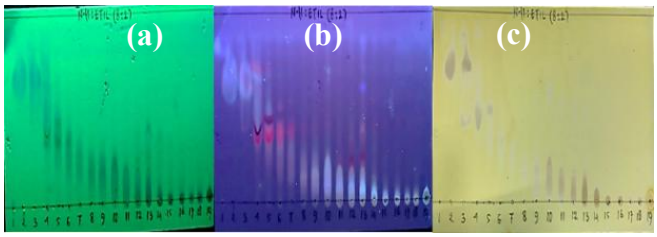


Figure 1. Stain profile of LVC results of EtOAc fraction of Isis sp. H:EtOAc (8:2) mobile phase at; (a) λ 254 nm; (b) λ UV 366 nm; (c) $CeSO_4$ stain.

Based on the thin-layer chromatography (TLC) stain profile derived from the liquid-vapor chromatography (LVC) results, the fractions were categorized according to their respective R_f values, resulting in a more streamlined classification of fractions. Specifically, fraction code A1 was assigned to the combination of stains 1-4, fraction code B1 to stains 5-8, fraction code C1 to stains 9-13, fraction code D1 to stains 14-16, fraction code E1 to stains 17-18, and fraction code F1 to stains 19-21. Furthermore, fraction code G1 was designated for the wash conducted with methanol (MeOH). The simplified fractions were subsequently re-dotted, and the combined TLC stain profile obtained from LVC-1 employing mobile phase H: ethyl acetate (EtOcA) in a 5:5 ratio is depicted in Figure 2.

Fraction A weighed 2.8387 g, Fraction B weighed 0.2538 g, Fraction C weighed 1.301 g, Fraction D weighed 0.7595 g, Fraction E weighed 1.545 g, and Fraction F weighed 3.1741 g. The total extract of *Isis sp.* utilized for liquid–vapor chromatography (LVC) was 150 g, and the cumulative weight of the fractions obtained from the fractionation process was 9.8721 g, as indicated in Table 1.

Antibacterial Activity Test Result

Antibacterial activity testing was conducted using the *Broth Microdilution* method to determine the Minimum Inhibitory Concentration (MIC) value. The MIC value is ascertained by evaluating the activity of the sample at its lowest concentration, which is diluted in a *microplate* containing both media and microbial inoculum. The MIC value can be visually observed through the formation of clear media or quantified using spectrophotometric methods, where absorbance values approaching zero indicate the absence of turbidity. The bacterial strains utilized in the antibacterial activity assessment include *Staphylococcus aureus* ATCC 25923, a gram-positive bacterium, and *Escherichia coli* ATCC 35218, a gram-negative bacterium. The selection of these bacterial strains is intended to evaluate the spectrum of activity of the tested fraction.

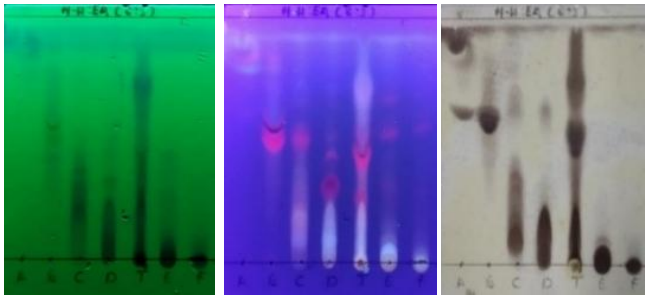


Figure 2. Combined TLC stain profile of LVC results of EtOAc fraction of *Isis* sp. H mobile phase: EtOAc (8:2) at; (a) λ 254 nm; (b) λ 366 nm; (c) $CeSO_4$ Stain Exposer.

The total extract of *Isis* sp. used for LVC was 150 g. the total fractionated extract was 9.8721 g. which can be seen in Table 1.

Table 1. Weight of fractions of combined LVC results

Faction Code	Fraction Weight (g)
A	2.8387
B	0.2538
C	1.301
D	0.7595
E	1.545
H	3.1741
Total	9.8721

The test results obtained were compared with positive control and negative control. Positive control is used with the aim of knowing the effectiveness of the sample tested. If the MIC value of the fraction formed is smaller than the positive control, the fraction is more effective as an antibacterial and vice versa. The negative control aims to prove that the solvent used to dissolve the sample has no effect (if active as an antibacterial) on its absorbance value. The positive control used is chloramphenicol while the negative control uses 5% DMSO solvent. Chloramphenicol was used because this drug has broad spectrum antibacterial activity which can inhibit Gram positive and Gram-negative bacteria. DMSO 5% was used because the nature of this solvent is able to dissolve compounds that are polar and non-polar where with a concentration of 5% DMSO is not able to inhibit cell growth (Galvao et al., 2014).

The test samples utilized in antibacterial testing included *Isis* sp. fractions A, B, C, D, E, and F. According to Andino-Molina et al., (2024) in their study on antimicrobial testing, the minimum inhibitory concentration (MIC) value categories are classified as strong if the MIC value is ≤4 µg/mL, intermediate if the MIC value is between 8-16 µg/mL, and weak or resistant if the value is ≥32 µg/mL. The results of the MIC value

measurements for the antibacterial testing of fractions A, B, C, D, E, and F are presented in Table 2.

Table 2. The results of measuring the MIC value of antibacterial fraction compounds against test bacteria (*Staphylococcus aureus* and *Eschricia coli*).

Sample	MIC value (µg/mL)	
	E.coli	*S. aureus*
Fraction A	8	128
Fraction B	4	32
Fraction C	8	64
Fraction D	64	128
Fraction E	2	2
Fraction F	8	64
Chloramphenicol	0.5	0.5

Table 2 presents the antibacterial activity of each fraction of *Isis* sp. soft coral against *Escherichia coli* and *Staphylococcus aureus*. Fractions B and E demonstrated strong activity against *Escherichia coli*, exhibiting minimum inhibitory concentration (MIC) values of 4 and 2 µg/mL, respectively. In contrast, fractions A, C, and F displayed intermediate activity, each with an MIC value of 8 µg/mL, while fraction D exhibited weak activity in inhibiting the growth of *Escherichia coli*, with an MIC value of 64 µg/mL. Furthermore, fraction E also demonstrated intermediate activity against *Staphylococcus aureus*, with an MIC value of 16 µg/mL, whereas fractions A, B, C, D, and F showed weak activity, with MIC values of 128, 32, 64, 128, and 64 µg/mL, respectively.

Chloramphenicol exhibits strong inhibitory activity against the test bacteria *Escherichia coli* and *Staphylococcus aureus*, with minimum inhibitory concentration (MIC) values of 0.5 µg/mL for both organisms. Jean et al., (2001) indicated that an extract possesses significant antibacterial activity if the MIC value against bacteria is ≤4 µg/mL.

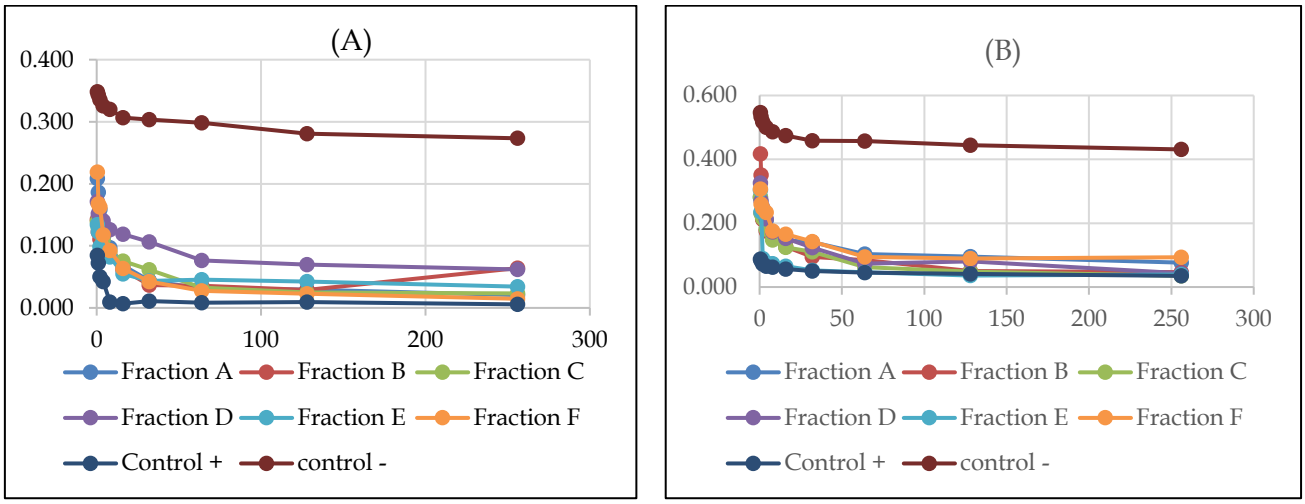


Figure 3. Antibacterial activity of samples against bacteria (A) *E. coli* and (B) *S. Aureus*

This finding aligns with the statement by Prasetia et al. (2022) that soft coral *Isis* sp. demonstrates antibacterial activity. Based on a structural perspective, chloramphenicol inhibits bacterial growth due to its ability to interact as an inhibitor of the peptidyl transferase enzyme located in the 50S ribosomal subunit of bacteria, thereby disrupting protein synthesis. The key structural elements contributing to the antibacterial activity of chloramphenicol include the chlorine atom, the aromatic ring with nitro group substituents in the para position, and two hydroxyl groups within the molecular framework.

Natural compounds that significantly contribute to antibacterial activity include phenolic groups (such as flavonoids, tannins, and stilbenes), alkaloids, and terpenoid compounds belonging to the monoterpene and diterpene classes. The compound identified in the soft coral *Isis* sp. in fraction E is a steroid group compound that exhibits antibacterial activity, with a minimum inhibitory concentration (MIC) of 2 µg/mL against each tested microbe, categorizing it as a strong antibacterial agent.

The structure of the compounds present in soft coral *Isis* sp. fraction E contains a hydroxyl group (OH), which plays a crucial role in antibacterial activity. This functional group is capable of damaging cell membranes and denaturing proteins, leading to compromised cell walls due to decreased permeability (Wang et al., 2024). Figure 3 shows that the greater the concentration given, the smaller the absorbance value produced. This also means that the activity of the compounds in the fraction of *Isis* sp. soft coral against the test bacteria *E.coli* and *S. aureus* increases as the concentration is increased. Based on the results of identification of secondary metabolite compounds using Liquid Chromatography Mass Spectroscopy/Mass Spectroscopy (LC-MS/MS)

obtained four compounds there are deoxyadenosine, spinasterol, pseudoionone, and isosalsoline. The structures of these compounds are illustrated in Figure 4.

ADME Test Analysis Results

There are 3 compounds out of 4 compounds isolated from *Isis sp.* soft coral that fulfill the Lipinski RO5 (Lipinski's rule of five) (Table 3). The parameters of the Lipinski RO5 are the initial stage in determining the oral bioavailability of active substances because they relate to the acceptance of solubility and permeability of compounds in living cells. The test compound must meet the Lipinski RO5 (Varma et al., 2006).

The test results in Table 3 show that of the 4 compounds isolated from *Isis sp.* soft coral, only 3 compounds fulfill the Lipinski RO5. Based on the test results, there are 3 compounds that can be continued for molecular docking test. These compounds are deoxyadenosine, pseudoinone and isosalsoline.

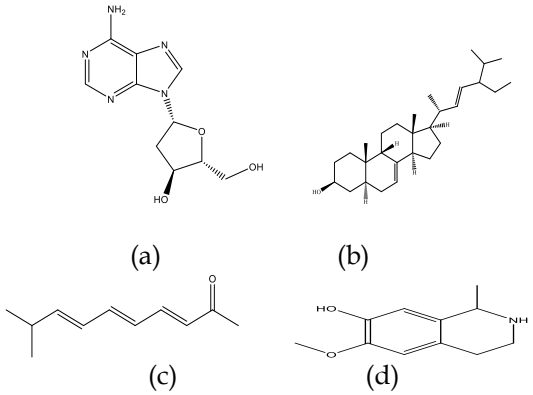


Figure 5. Structure of compounds (a) Deoxyadenosine. (b) Spinasterol. (c) Pseudoinone and (d) Isosalsoline

Table 3. of ADME Prediction Results According to Lipinski RO5

Compounds	Molecular Weight <500 g/mol	Hydrogen Bond Acceptor <10	Hydrogen Bond Donor <5	Lipophilicity (Log P <5)	Bioavailability
Deoxyadenosine	251.24	6	3	1.03	0.55
Spinasterol	412.69	1	1	5.02	0.55
Pseudoinone	192.30	1	0	3.82	0
Isosalsoline	193.24	3	2	2.27	0.55

Table 4. Binding energy, number of hydrogen bonds, and amino acids interacting with compounds in each binding sites of target proteins.

Compound	Docking Result	Target Protein (PDB ID)					
		5BNM	1AOE	4NC7	6M1S	1S14	6KVS
Deoxyadenosine	Binding energy (kcal/mol)	-2.868	-7.078	-2.993	-7.087	-6.215	-5.501
	H-bond	2: Arg-A:196, Val-A:197	2: ASP-A:75, ARG-A:78	no	3: ASP-A:75, ARG-A:78, GLY-A:79	3: ARG-A:1072, GLU-A:1046, ASP-A:1049	2:ALA-B:553, ASN-B;554
Pseudoinone	Binding energy (kcal/mol)	-6.164	-5.888	-2.572	-5.859	-5.465	-6.880
	H-bond	1: GLY-A:209	2: Arg-A:79, GLY-A:79	1: ASN-A:37	2: Arg-A:78, GLY-A:79	no	2:SER-B:583, CYS-B:425
Isosalsoline	Binding energy (kcal/mol)	-7.381	-5.967	-3.120	-5.979	-5.685	-5.354
	H-bond	2: ARG-A:36, ASN-A:247	1: GLU-A:52	2: LYS-A:47, GLU-A:34	1: GLU-A:52	no	1:CYS-A:452
Native ligand	Binding energy (kcal/mol)	-8.907	-11.627	-2.736	-8.858	-7.621	-6.901
	H-bond	4: ASN-A:274, ASN-A:247,	9: ALA-A:11, ILE-A:19, ALA-A:155, GLY-A:114, ILE-A:117, GLU-	1: ASN-A:37	1: ARG-A:138	4: ARG-A:1132, ASP-A:1077,	2:ASN-B:581, SER-B:583

Compound	Docking Result	Target Protein (PDB ID)					
		5BNM	1AOE	4NC7	6M1S	1S14	6KVS
		HYS-A:244, CYS-A:112	A:116, SER-A:80, SER-A:94, GLU-A:120			ASN-A:1042, ASP-A:1069	
	RMSD (Å)	0.250	0.524	1.499	1.757	0.553	1.592

Docking Results Analysis

The docking experiments conducted against six target proteins were designed to investigate the interactions responsible for antibacterial activity and to identify the most dominant type of protein. Validation of the docking procedures was performed by re-docking each of the reference ligands onto their respective binding sites, with re-docking accepted if the < 2.0Å (Yousif et al., 2025). In binding site of *E. coli* (PDB:1S14), the test ligands didn't form hydrogen bonds and their interaction patterns were different. This evidence inferred the binding mode of test compounds were not same as chloramphenicol. Docking study exploring the conformation of all docked molecules within the binding site of *S. aureus* (PDB:6KVS) showed that ligands confined within the binding site and take similar conformation with reference ligand (Color: Blue, pink, and yellow) (Figure 6).

The results of the compound binding site analysis for deoxyadenosine, pseudoinone, and isosalsoline, which exhibited the most favorable binding energy, provide an overview of the hydrogen bonds formed. Deoxyadenosine forms three hydrogen bonds with arginine (ARG-A: 78), aspartate (ASP-A: 75), and glycine (GLY-A: 79). Pseudoinone forms one hydrogen bond with glycine (GLY-A:GLY). Isosalsoline forms two hydrogen bonds, one with arginine (ARG-A:36) and one with asparagine (ASN-A:247). The optimal interaction results between the natural ligands and receptor proteins are illustrated in Figure 7. It can be concluded that these compounds exhibit a similar binding mode to that of the reference ligand (Reshma et al., 2025).

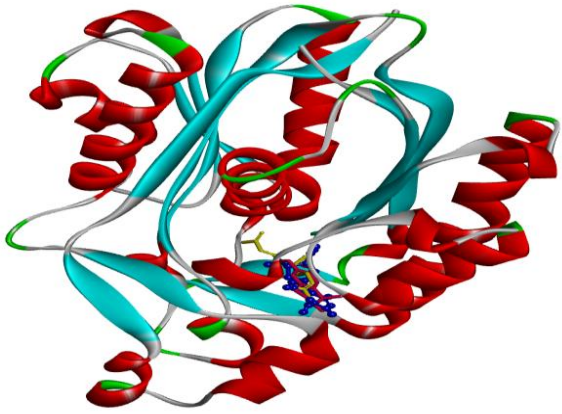
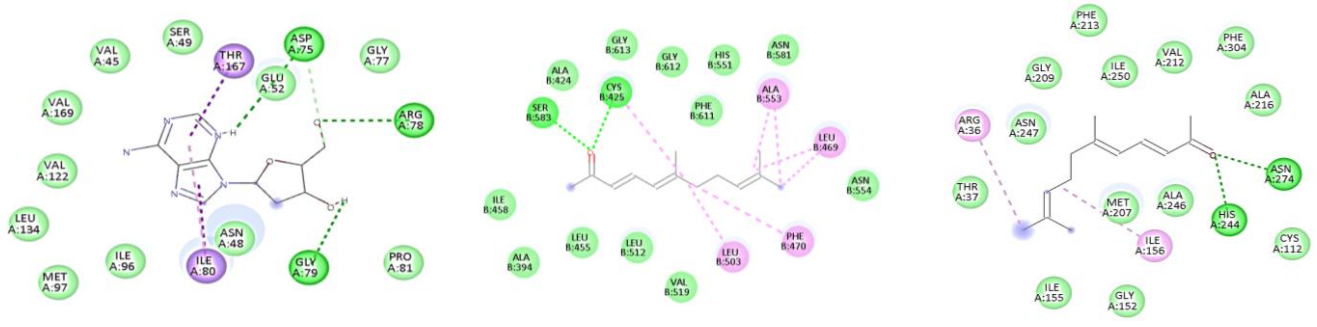


Figure 6. Docked conformation of test compounds in binding site (cavity-1) of *S. aureus* (PDB:6KVS).



A B C

Figure 7. Docking interactions with amino acids (A) deoxyadenosine-(PDB.6M1S), (B) Pseudoinone-(PDB: 6KVS), (C) Isosalsoline-(PDB: 5BNM). Green dashed-line denoted H-bond, purple circle is covalent bond, pink dashed-line pointed π-alkyl interaction

To select the possible interaction mechanism underlying the antibacterial activity, the following criteria were used: (1) the RMSD of the compound assay should be less than the RMSD of the reference ligand, (2) the compound forms a hydrogen bond with the amino acid at the binding site, (3) the interacting amino acid is the same as the reference ligand which means the compound has the same binding mode, (4) high binding energy which has similarity with the MIC of antibacterial activity (Khalaf et al., 2025). Based on these requirements, the most suitable pattern was found that the deoxyadenosine compound has a good interaction with *P. aeruginosa gyrase* (PDB. 6M1S), this is supported by the best binding energy (-7.087 kcal/mol). Pseudoinone compounds have good interactions with *S. aureus* (PDB: 6KVS), this is supported by the best binding energy (-6.880 kcal/mol). Isosalsoline compounds have

good interactions with *E. coli* (PDB: 5BNM), this is supported by the best binding energy (-7.381 kcal/mol).

Based on these results, it shows that each isolated compound has a different ability to fight bacteria. When referring to the results of in vitro antibacterial tests that have been carried out and data from molecular docking tests, it can be predicted that compounds isolated from *Isis sp*. soft coral, namely pseudoinone compounds have a role as anti-bacterial *S. aureus* (PDB: 6KVS). This compound also shows a strong ability as an anti- gram-positive bacterium than gram negative. Isosalsoline compounds have a role as anti-bacterial *E. coli* (PDB: 5BNM). This compound also shows a strong ability as an anti-gram-negative rather than gram positive (Hu et al., 2025).

Conclusion

Secondary metabolite compounds that can be identified from the ethyl acetate extract fraction of *Isis* sp. soft coral using LC (*Liquid Chromatograph*) obtained the highest BPI at RT 1.16. 13.6. 1.03 and 1.71 minutes. then validated with MS/MS (Mass Spectrometry/Mass Spectrometry) it was known that the four compounds were consecutively LC-MS/MS including Deoxyadenosine ($C_{10}H_{13}N_5O_{3)}$, Spinasterol ($C_{29}H_{48}O$), Pseudoinone ($C_{11}H_{16}O$) and Isosalsoline ($C_{11}H_{15}NO_{2)}$. The results of the antimicrobial activity test of fraction E *Isis* sp. showed strong activity against *S.aureus* bacteria and *E. coli* bacteria with a MIC value of 2 µg/mL.

While the activity against the test fungi *C. albicans* was strong at the 24th hour. intermediate at the 48th hour and weak at the 72nd hour with MIC values of 4. 16 and 64 µg/mL respectively. Based on molecular docking analysis can be predicted that compounds isolated from *Isis* sp. soft shells, namely pseudoinone compounds have a role as anti-bacterial *S. aureus* (PDB: 6KVS). This compound also shows a strong ability as an anti- gram positive bacteria than gram negative. Isosalsoline compounds have a role as anti-bacterial *E. coli* (PDB: 5BNM). This compound also shows a strong ability as an anti-gram negative bacteria rather than gram positive.

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The authors declare no conflict of interest

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