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Exploring *Polycarpa aurata* Quoy and Gaimard, 1834 Extracts as Antibiotic Candidates: GC-MS Profiling and Molecular Docking Study

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ABSTRACT

Marine natural products have garnered global interest due to their remarkable bioactive compounds. The tunicate *P. aurata*, a highly abundant marine invertebrate, possesses significant bioactive potential with applications as an anti-cancer, antibacterial, and antifungal agent. This study aims to evaluate the potential of *P. aurata* bioactive compounds as antibiotic candidates through *in vitro* testing and to explore their activity via an *in-silico* approach using molecular docking. *P. aurata* was extracted using maceration, yielding both methanol and *n*-hexane extracts. The methanol extract of *P. aurata* demonstrated greater efficacy than the *n*-hexane extract, particularly against *Staphylococcus aureus* with an inhibition zone diameter of 18.8 mm, compared to 13 mm for *Salmonella typhi*, both at a 25% concentration. In comparison, the positive control, ciprofloxacin, produced an inhibition zone ranging from 22 to 24 mm for both bacterial strains. GC-MS analysis of the extract revealed three compounds with high % area and similarity index values: Cyclohexane, 1,3,5-triphenyl, Cholesta-5,22-dien-3-ol, and Cholesta-3,5-diene, all of which were suitable for the selected protein target. Computational analysis through molecular docking demonstrated that these compounds exhibit stronger binding affinities compared to ciprofloxacin. This study suggests that the extract of *P. aurata* is a promising source of bioactive compounds with substantial therapeutic potential as an antibacterial and antibiotic candidate.

1. Introduction

The ocean is one of Earth's most diverse and complex ecosystems, offering a vast array of biodiversity with significant potential for human benefit (Zamani *et al.* 2021). In the search for novel therapeutic agents, marine natural products (MNPs) have gained considerable attention due to their unique bioactive compounds. Certain marine organisms produce specialized chemicals with applications across various industries, including pharmaceuticals, nutraceuticals, molecular probes, and more (Litaay *et al.* 2018; Karthikeyan *et al.* 2022; Sibero *et al.* 2022). Among these organisms, the tunicates (phylum Tunicate) are particularly notable for their abundance and the diversity of bioactive compounds they produce, positioning them as a valuable resource for biotechnological and pharmaceutical research (Scarabino *et al.* 2018; Ramesh *et al.* 2021).

The phylum Tunicata comprises approximately 3,000 species classified into four main groups: *Ascidiacea, Sorberacea, Thaliacea*, and *Appendicularia*. Tunicates are notable for their invasive tendencies and rapid growth rates, making them a prominent group within marine ecosystems (Atmaca *et al.* 2013; Santhanam & Ramesh 2020; Litaay *et al.* 2023). Beyond their ecological roles, tunicates are valued for their capacity to produce a range of bioactive compounds with potential applications in

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biotechnology and medicine (Ramesh *et al.* 2021; Sibero *et al.* 2022). Although numerous species within Tunicata exhibit bioactivity, only about 12% of these compounds have been identified from the family Styelidae. One notable member of this family, *P. aurata*, is particularly abundant and distinguishable by its vibrant tunic colors (Hanif *et al.* 2019; Lestari *et al.* 2022; Gao *et al.* 2023).

P. aurata is of special interest due to its antimicrobial properties, including activity against Methicillin-resistant *S. aureus* (MRSA). This species produces rare bioactive compounds, such as polycarpine, polycarpaurine, polycarpathiamines A and B, as well as polyaurins A and B-metabolites seldom found in other natural sources (Pham *et al.* 2013; Casertano *et al.* 2019; Ruli & Tapilatu 2020). These compounds have demonstrated various therapeutic activities, including antibacterial, anti-cancer, and antifungal properties, with some even showing efficacy in treating soft tissue sarcoma (Sinko *et al.* 2012; Sardiani *et al.* 2015; Shaala & Youssef 2015; Litaay *et al.* 2019a). Despite these promising attributes, research on the specific bioactive compounds of *P. aurata* from the Sangkarang Islands region remains limited, underscoring the need for further exploration.

Bioactive compound discoveries from natural sources have intensified as the demand for new medicinal agents grows, particularly in response to the global rise in multidrug-resistant infections. This continuous exploration aims to discover novel therapeutic compounds to combat resistance that has developed from the repeated use of conventional drugs, such as antibiotics (Casertano *et al.* 2019; Zamani *et al.* 2021). Antibiotics are widely utilized to treat various infectious diseases; however, the rapid emergence of antibiotic-resistant pathogens poses a significant global health threat. This resistance diminishes the effectiveness of treatments, leading to prolonged and costlier medical interventions and heightening the challenge of disease management (CDC 2019; Murray *et al.* 2022; Buniya *et al.* 2024).

Research on the antibacterial activit y of tunicate *P. aurata* extract has been conducted *in vitro* in several studies. However, these studies are still largely limited to computational analysis-based approaches using bioinformatics, such as molecular docking methods. The development of new drugs, including antibiotics, necessitates an early evaluation of their effectiveness and potential toxicity, which can be facilitated by computational bioinformatics techniques, such as *in silico* analysis. This method is used to predict and understand the mechanism of activity and interaction between bioactive compounds and target proteins, including binding and affinity to specific proteins or receptors (Prito-Martinez *et al.* 2018; Wardhani *et al.* 2024). This study aims to assess the antibiotic potential of bioactive compounds from *P. aurata* through vitro assays. *Salmonella typhi* and *Staphylococcus aureus* were chosen as test organisms due to their clinical relevance, pathogenicity, and antibiotic resistance challenges, making them ideal models for evaluating potential antibiotics. Ciprofloxacin, a broad-spectrum antibiotic with proven efficacy and stability, was used as a positive control to assess the antibacterial activity of the bioactive compounds objectively. Additionally, it seeks to profile the metabolite compounds of *P. aurata* using gas chromatography-mass spectrometry (GC-MS) and Fourier-transform infrared spectroscopy (FTIR) and to further characterize these compounds via a computational bioinformatics approach using silico methods.

2. Materials and Methods

2.1. Sampling

Specimens of the tunicate *P. aurata* were collected from the waters surrounding Barranglompo Island, Makassar, South Sulawesi (119°19'48“EL and 05°02'48” SL). Samples were located at depths of 2-10 meters, and collection was conducted using scuba diving techniques. Individual *P. aurata* specimens, measuring approximately 5-7 cm, were carefully harvested by cutting them at the base attached to the substrate, with larger specimens being selected for study (Selviati *et al.* 2024). The samples were thoroughly cleaned to remove any associated organisms and rinsed with distilled water. Following cleaning, the specimens were air-dried and subsequently freeze-dried to prepare them for the extraction process.

2.2. Isolation and Extraction of Bioactive Compounds

The dried *P. aurata* samples were ground into a fine powde r (100 mesh) and subjected to bioactive compound extraction through maceration, using methanol and *n*-hexane as solvents in a 1:3 (powder-to-solvent) ratio. The mixture was left to stand for 24 hours, following the methods described (Sinko *et al.* 2012; Litaay *et al.* 2019b). To enhance extraction efficiency, the process was repeated three times. The combined macerates were then concentrated using a rotary evaporator to produce a viscous/crude extract.

2.3. Antibacterial Activity Assay

Concentrated extracts of the tunicate *P. aurata* were prepared at concentrations of 15%, 20%, and 25%. Antibacterial activity was assessed using the disk diffusion method (Litaay *et al.* 2019a), whereby paper disks impregnated with the extract were placed on Mueller Hinton Agar plates inoculated with the test bacterial suspension. The plates were then incubated for 24 hours at 37°C, after which the antibacterial efficacy was determined by measuring the inhibition zones formed around the disks .

2.4. Identification of Metabolite Compounds

The secondary metabolite content of the *P. aurata* extract was analyzed using gas chromatography-mass spectrometry (GC-MS) and Fourier-transform infrared (FTIR) spectroscopy.

2.4.1. Fourier Transform Infrared Analysis (FTIR)

Samples were prepared by grinding 250 mg of potassium bromide (KBr) and pressing it into a pellet mold to produce a KBr pellet. The dried extract was then analyzed over a frequency range of 4000-400 cm^{-1}. FTIR provided qualitative data on the presence of specific functional groups and bond types at wavelengths, along with quantitative data based on the absorbance of detected functional groups (Nautiyal & Dubey 2021). Compound groups were analyzed using a Shimadzu FTIR instrument.

2.4.2. Gas Chromatography-Mass Spectrometry Analysis (GC-MS)

The *P. aurata* methanol extracts were analyzed using a Shimadzu GC-MS Ultra QP 2010 system. A 100 µL aliquot of each extract was injected into the chromatographic column under the following conditions: an injector temperature of 250°C in splitless mode, a pressure of 76.9 kPa, a flow rate of 14 ml/min, and a split ratio of 1:10. The ion source and interface temperatures were set at 200°C and 280°C, respectively. The solvent cut-off time was established at 3 minutes, and mass spectra were recorded over a range of m/z 400 to 700.

The analysis was carried out on an SH-Rxi-5Sil MS column, measuring 30 m in length with an internal diameter of 0.25 mm. The temperature programming started at 70°C with a 2-minute hold, followed by a ramp to 200°C at 100°C/min, and then a further increase to 280°C at 50°C/min. The total run time for the analysis was 36 minutes. Peak identification was performed by comparing the chromatographic data with the NIST 17 and Wiley 9 spectral libraries (Sivarajan *et al.* 2019). GC-MS is a technique that is often used in analysis to identify bioactive compounds. GC-MS experiments produce data that can be seen from chromatogram peaks as identification data from the measurement of metabolite abundance in samples in chromatography and mass spectrometry (MS) seen from mass spectra with each molecular weight of bioactive compounds (Zaraswati *et al.* 2024).

2.5. Molecular Docking

The *in-silico* approach utilizes computational methods to simulate and evaluate biological and chemical activities, aiding research areas such as drug development and genomics. Molecular docking was employed to assess the interactions between compounds from *P. aurata* (ligands) and bacterial target proteins (macromolecules). The chemical compounds used as ligands were identified through GC-MS analysis of *P. aurata* methanol extracts, and their corresponding SMILES IDs were retrieved from the PubChem database (https://pubchem.ncbi.nlm.nih.gov/). Target protein structures were obtained from the Protein Data Bank (PDB) (www.rscb.org/). To prepare the protein structures, PyMOL version 1.7.4.5 was used to remove non-protein entities (Husain & Wardhani 2021). The selected target proteins for this study were sortase-A from *S. aureus* (PDB ID: 1T2P) and DNA gyrase A from *S. typhi* (PDB ID: 5ZTJ). Protein preparation was carried out using Chimera (Pettersen *et al.* 2004), which included the addition of hydrogen atoms and the optimization of charges using AM1-BCC atomic. The molecular docking process was conducted using AutoDock Vina, integrated with the PyRx 0.8 platform. Visualization and analysis of ligand-protein interactions were performed using Discovery Studio version 21.1.0.20298 (Sari *et al.* 2024).

3. Results

3.1. Sampling

The identification and sampling of *P. aurata* were conducted based on morphological characteristics. Tunicates, including *P. aurata*, are commonly found in deeper marine environments, where they live attached to coral substrates. Due to their habitat, the sampling process required scuba diving techniques to ensure accurate collection.

3.2. Isolation and Extraction of Bioactive Compounds

The extraction of *P. aurata* samples was conducted using the maceration method. A total of 250 g of powdered sample was immersed separately in methanol and *n*-hexane solvents for 72 hours at room temperature, with the solvent being replaced every 24 hours. Methanol and *n*-hexane were selected due to their complementary polarities, enabling the extraction of a broader range of bioactive compounds. Methanol is particularly effective in extracting polar compounds, whereas *n*-hexane is suitable for nonpolar compounds. The extraction yields are summarized in Table 1. The extraction results show that methanol produced a higher yield (15 g, 7.5%) of a brownish-black extract compared to *n*-hexane, which yielded 2.5 g (1.25%) of a reddish-yellow extract. The higher yield with methanol reflects its effectiveness in extracting polar bioactive compounds, while the lower yield with n-hexane aligns with its suitability for nonpolar compounds. The distinct colors of the extracts suggest differences in chemical composition, with methanol likely extracting darker polar compounds, such as phenolics, and *n*-hexane isolating lighter nonpolar compounds, such as carotenoids.

3.3. Antibacterial Activity

The results of antibacterial activity testing against pathogenic bacteria are presented in Table 2. Additionally, Figure 1 illustrates the inhibition zones formed by each extract at concentrations of 15%, 20%, and 25%. The results demonstrate that the methanol extract of *P. aurata* exhibits higher antibacterial activity than the *n*-hexane extract against both *S. aureus* and *S. typhi*. The inhibition zones for the methanol extract increased with concentration, reaching a maximum of 18.8 mm for *S. aureus* and 13.8 mm for *S. typhi* at 25% concentration . In contrast, the *n*-hexane extract displayed minimal activity, with inhibition zones not exceeding 8.2 mm for either bacterium, even at the highest concentration. The positive control, ciprofloxacin, produced significantly larger inhibition zones, confirming its effectiveness as a broad-spectrum antibiotic.

Table 1. Annotation and characteristics of the PLCs in cocoa

Sample	Ratio (sample: solvent)	Yield (g)	Yield (%)	Extract color
Methanol extract	1:3	15	7.5	Brownish Black
n-hexane extract	1:3	2.5	1.25	Reddish Yellow

3.4. Identification of Metabolite Compounds

The extraction results indicate that the methanol extract yielded a higher quantity of extract and exhibited stronger antibacterial activity compared to the *n*-hexane extract (Table 2). Consequently, the methanol extract was selected for further metabolite characterization and identification using Fourier Transform Infrared Spectroscopy (FTIR) and Gas Chromatography-Mass Spectrometry (GC-MS).

3.4.1. FTIR Analysis

The FTIR results of the sample's wave number and functional group can be seen in Table 3. The FTIR analysis of the methanol extract of *P. aurata* revealed the presence of diverse functional groups. Key peaks include 3419 cm^{-1} (O-H, alcohol), 3246 cm^{-1} (N-H, amine), and 1639 cm^{-1}, 1510 cm^{-1} (C=C, aromatic), indicating the presence of alcohols, amines, and aromatic compounds. The peaks of 1411 cm^{-1} (CH_2, alkane) and 1253 cm^{-1} (C-N, aromatic amine) further highlight their structural diversity. The bands at 1176 cm^{-1} and 1045 cm^{-1} represent C-O stretching (ether and alcohol), while 623 cm^{-1} and 516 cm^{-1} suggest C-X (halogen). These functional groups reflect the polar nature of methanol, facilitating the extraction of bioactive compounds.

3.4.2. Identified Compounds Using GC-MS

The chromatograms of the methanol extract are presented in Figure 2. The GC-MS analysis of *P. aurata* methanol extracts identified 97 compounds, with Cyclohexane, 1,3,5-triphenyl being the most abundant, showing an area retention of 39.60% (A). This compound, also known as 1,3,5-triphenylcyclohexane, is an organic molecule

Table 2. Antimicrobial activity of the *P. aurata* extracts

Treatment	Concentration (%)	Inhibition zone (mm)	
		S. aureus	*S. thypi*
Methanol extract	15	15.3	13
	20	16.2	12.5
	25	18.8	13
	Ciprofloxacin (+)	23.5	24
n-hexane extract	15	6.3	6.1
	20	6	6.2
	25	6.8	6.2
	Ciprofloxacin (+)	22.5	22

from the benzene derivative class with a complex structure comprising three phenyl rings attached to a cyclohexane core, which confers unique physical and chemical properties. Other significant compounds include 1-Octadecanol (5.01%) (B), 9-Octadecenoic acid (Z)-, methyl ester (4.37%) (C), 1,1-Diphenylcyclopropane (4.35%), and Hexadecanoic acid, methyl ester (3.83%), along with numerous other compounds of varying concentrations, collectively indicating a diverse chemical profile in the methanol extract.

3.5. Molecular Docking

Molecular docking analysis was carried out on three compounds with the largest % area found in Methanol extract *P. aurata*. Molecular docking analysis is performed using AutoDock4 software with the help of AutoDockTools. Table 4 presents the binding energy results of the chemical compounds identified in the methanol extract of *P. aurata* against *S. aureus* Sortase A and *S. typhi* DNA gyrase proteins, as determined through molecular docking. Three compounds-cyclohexane, 1,3,5-triphenyl, cholesta-5,22-dien-3-ol, and cholesta-3,5-diene exhibited notable binding energies, indicating potential interactions with the target proteins (Figures 3 and 4). Among these, cholesta-3,5-diene showed the lowest binding energy values for both Sortase A (-8.65 kcal/mol) and DNA gyrase (-7.92 kcal/mol), suggesting

Table 3. The compounds identified in the methanol extract of the tunicate *P. aurata* using FT-IR analysis

Functional group	Wavelength (cm^{-1})
O-H (Alcohol)	3419
N-H (Amine)	3246
C=C (Aromatic)	1639 and 1510
CH2 (Alkane)	1411
C-N (Aromatic amine)	1253
C-O (Eter)	1176
C=C (Alkene)	1045
C-X (Halogen)	623 and 516

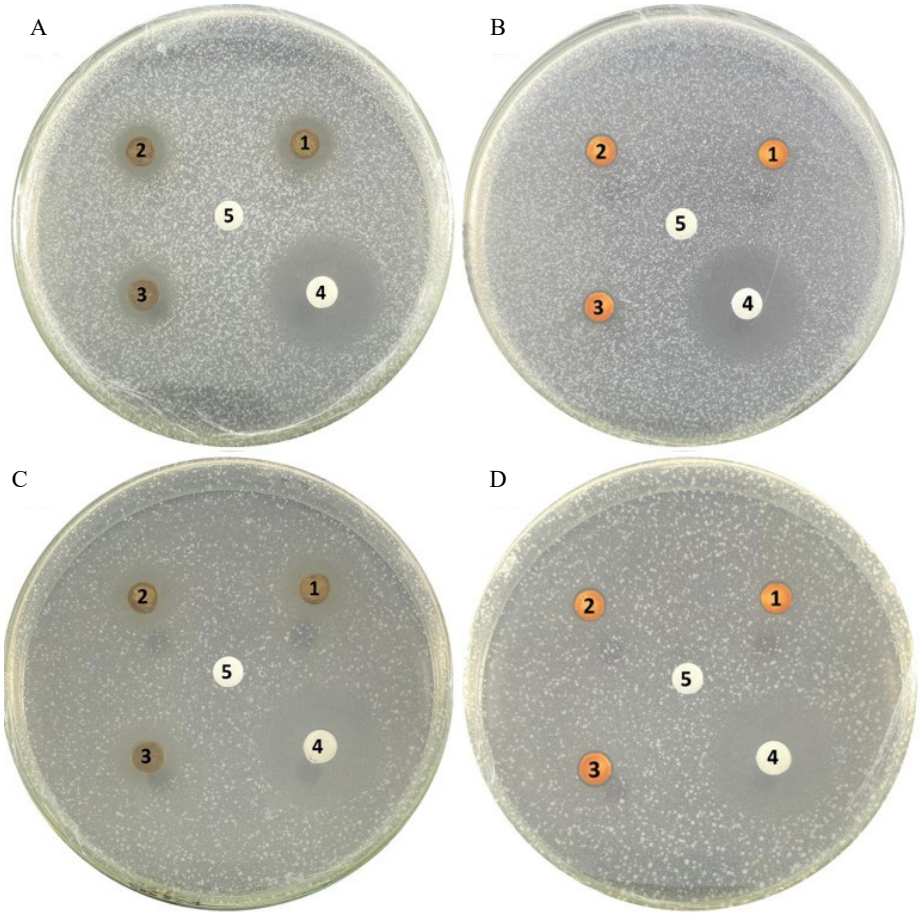

Figure 1. Antibacterial activity of *P. aurata* extracts: (A) Methanol extract against *S. aureus*, (B) *n*-hexane extract against *S. aureus*, (C) methanol extract against *S. typhi*, (D) *n*-hexane extract against *S. typhi*. The labels indicate: (1) 15% extract concentration, (2) 20% extract concentration, (3) 25% extract concentration, (4) positive control (Ciprofloxacin), and (5) negative control (Solvent)

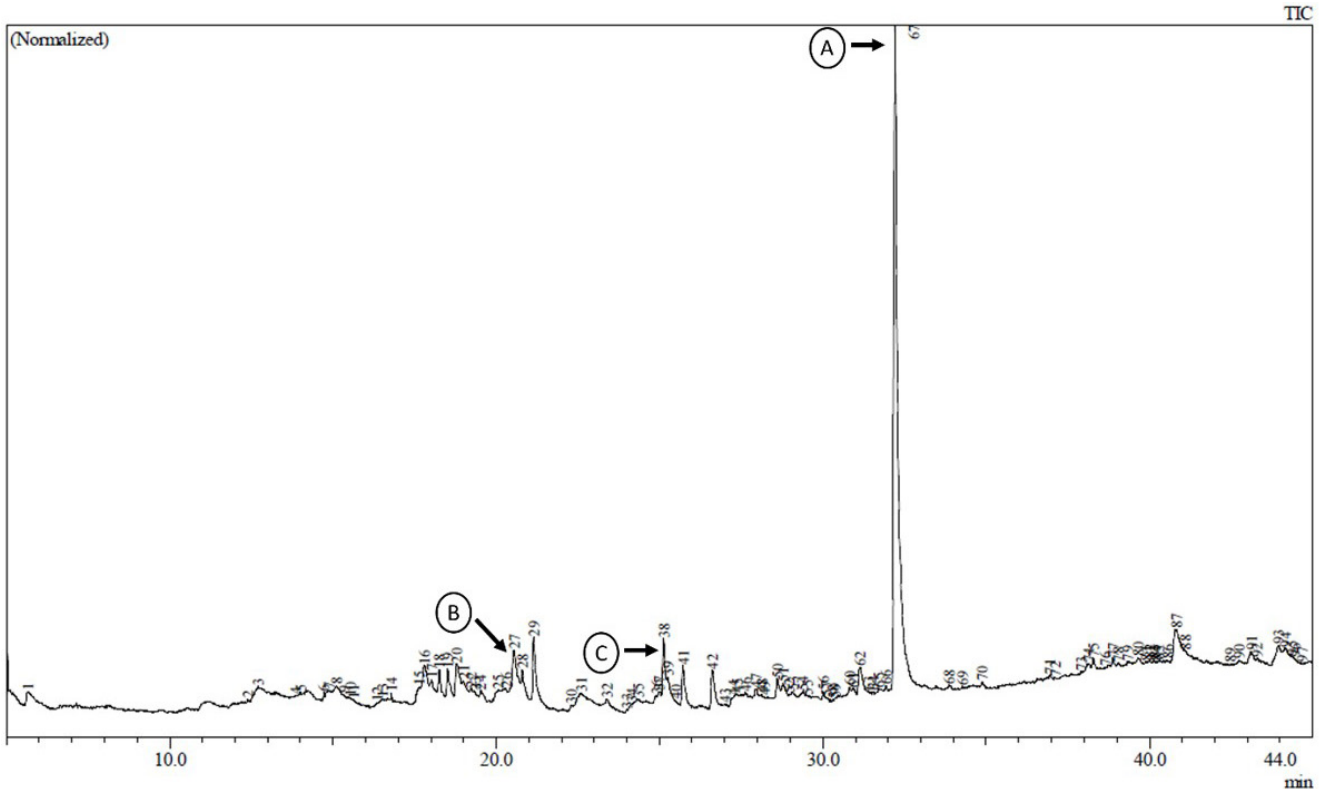

Figure 2. Chromatogram of *P. aurata* methanol extract

Table 4. Results of the binding of compound molecules contained in Methanol *P. aurata* extract to *S. aureus* sortase-A and DNA gyrase receptor protein from *S. thypi*

PubChem ID	IUPAC name	Binding energy (kcal/mol)	
		Sortase A	DNA gyrase
119930	Cyclohexane, 1,3,5 Triphenyl	-7.36	-7.46
5283661	Cholesta-5,22-Dien-3-ol	-8.22	-7.54
92835	Cholesta-3,5-Diene	-8.65	-7.92
2764	Ciprofloxacin	-7.3	-6.88

it may have the strongest binding affinity. Similarly, cholesta-5,22-dien-3-ol demonstrated significant binding energy values (-8.22 kcal/mol for Sortase A and -7.54 kcal/mol for DNA gyrase). Cyclohexane, 1,3,5-triphenyl displayed slightly higher binding energy values, indicating comparatively weaker interactions. Ciprofloxacin, the positive control, exhibited binding energies of -7.3 kcal/mol and -6.88 kcal/mol for Sortase A and DNA gyrase, respectively.

4. Discussion

Barrang Lompo Island is renowned for its coral reef ecosystem, which supports a rich diversity of marine organisms, including tunicates such as *P. aurata* (Abdurrachman *et al.* 2024; Lapong *et al.* 2024). This marine invertebrate, with its sedentary lifestyle, is commonly found attached to coral reefs in shaded or deeper waters. *P. aurata* is distinguished by its urn-shaped body, two yellow siphons, and a predominantly white tunic with blue and yellow stripes (Litaay *et al.* 2023). The highest yield of *P. aurata* extract was obtained using methanol, indicating that the bioactive compounds in *P. aurata* are predominantly polar. Polar solvents, such as methanol, typically produce higher extraction yields compared to nonpolar (Ekka & Owary 2023). A higher extract yield generally signifies the successful extraction of more bioactive substances from the raw material (Selviati *et al.* 2024).

The extracts obtained were tested for their biological activity against *S. aureus* and *S. typhi*. The antibacterial activity was observed as a clear area surrounding the disc paper treated with the extract (Lestari *et al.* 2022; Sibero *et al.* 2022). The methanol and *n*-hexane extract of *P. aurata* exhibited the largest inhibition zone at a 25% concentration. The antibacterial activity of the *P. aurata* extracts suggests that the compounds within the extract can inhibit or kill bacteria (Husain & Wardhani 2021; Husain *et al.* 2022; Wardhani *et al.* 2024). The

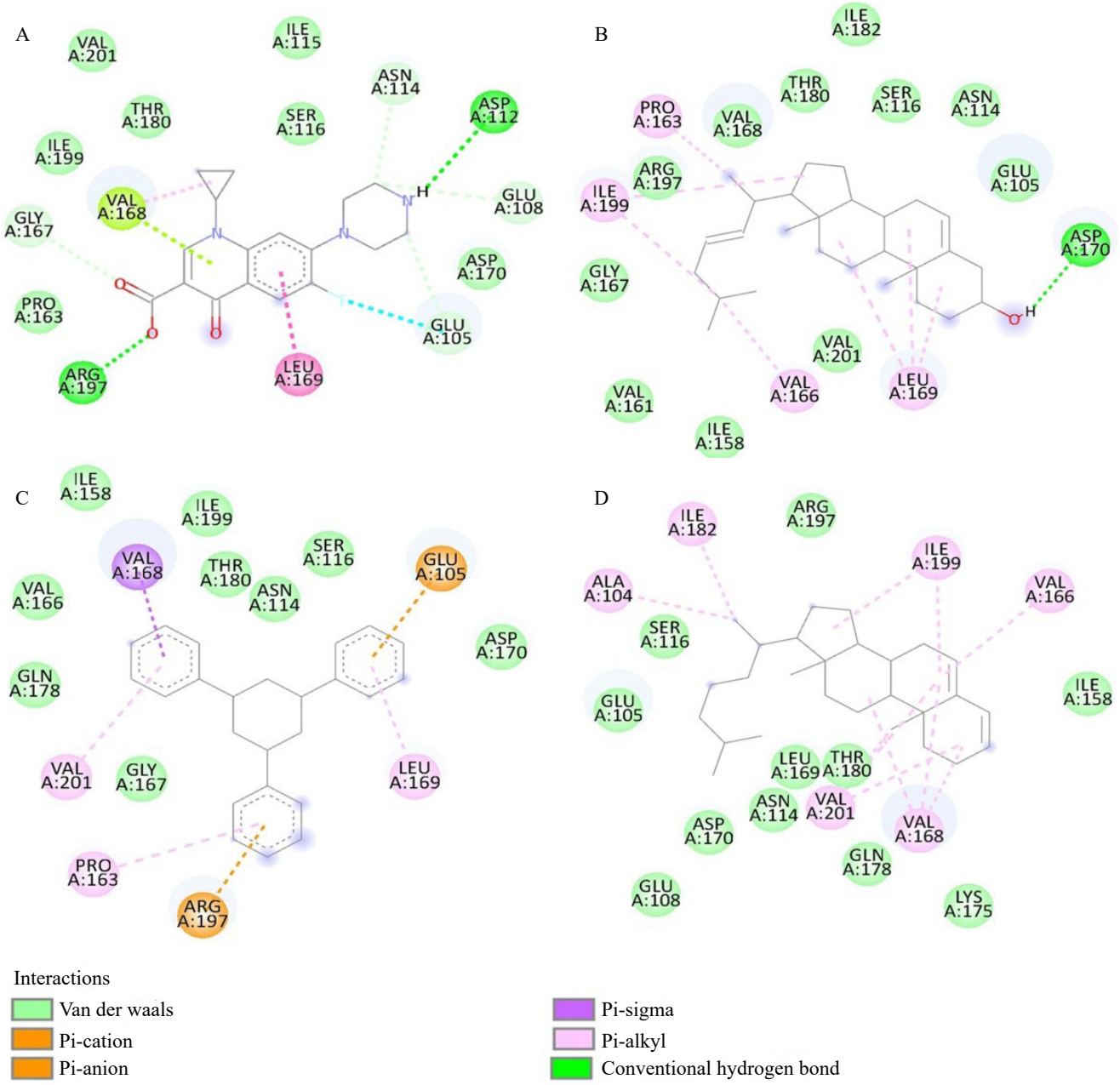

Figure 3. 2D interactions of (A) ciprofloxacin, (B) Cyclohexane, 1,3,5 Triphenyl, (C) Cholesta-5,22-Dien-3-ol, and (D) Cholesta-3,5-Diene to *S. aureus* sortase-A

difference in antibacterial activity between methanol and *n*-hexane extracts can be attributed to the differing properties of these solvents. Methanol is effective in extracting moderately polar compounds, such as alkaloids, flavonoids, and steroids. In contrast, *n*-hexane, a nonpolar solvent, dissolves lipophilic compounds, such as alkanes, fatty acids, sterols, terpenoids, and alkaloids (Tamasi *et al.* 2021). The compounds in *P. aurata* extract show the potential to inhibit drug-resistant bacteria and could be used synergistically to address antibiotic resistance (Litaay *et al.* 2019a; Li *et al.* 2022).

The FT-IR spectrum reveals key absorption bands at 3419 cm^{-1} and 3246 cm^{-1}, corresponding to hydroxyl (O-H) and amine (N-H) groups, respectively. These findings confirm the presence of alcohol and amine functional groups in the extract, which align with the chemical profiles identified through GC-MS analysis. The GC-MS results detected secondary metabolites, including alkaloids, terpenoids, steroids, and fatty acids, known for their antibacterial and anti-inflammatory properties (Karthikeyan *et al.* 2022). Among these, Cholesta-5,22-

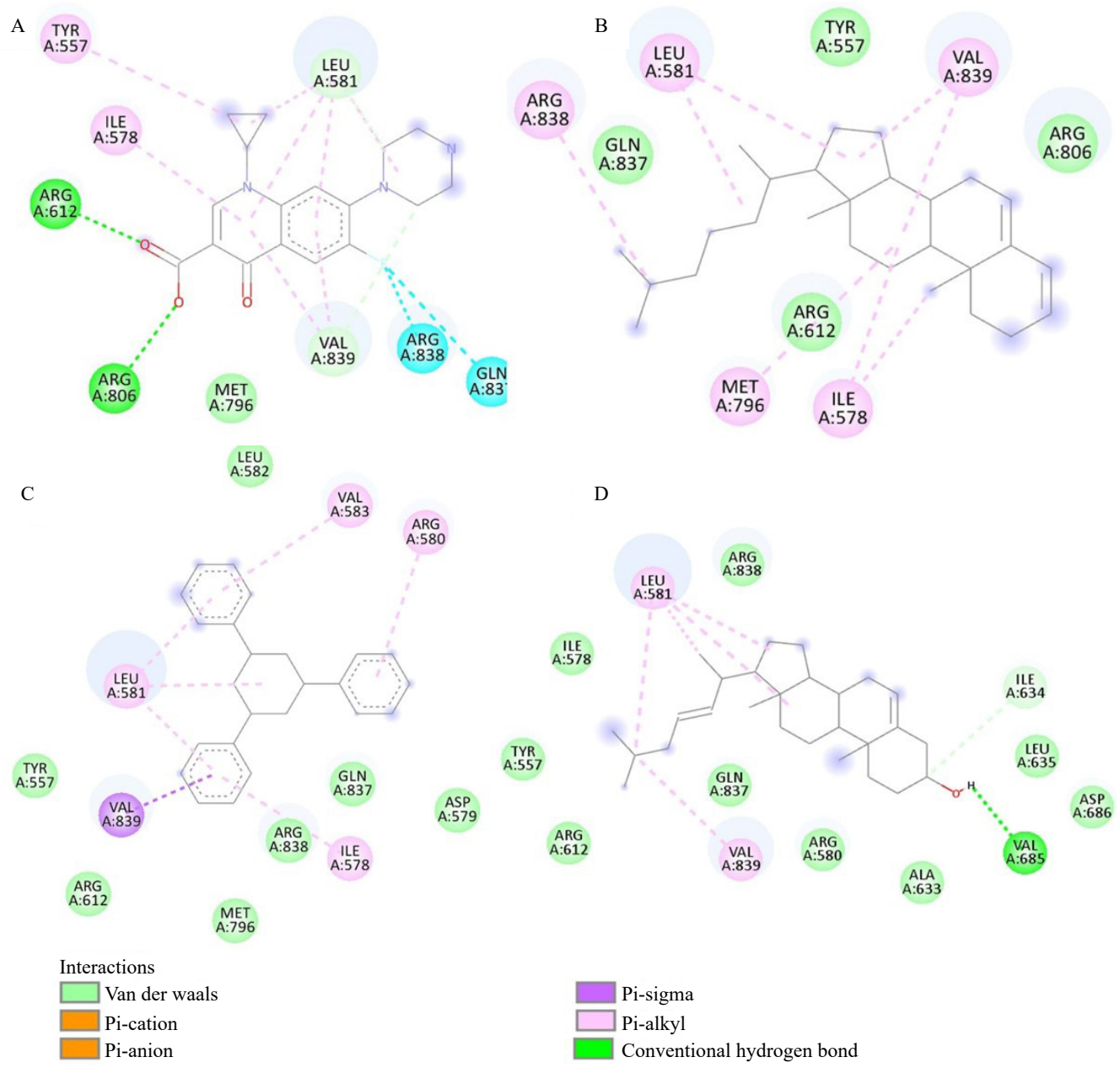

Figure 4. 2D interactions of compounds (A) ciprofloxacin, (B) Cyclohexane, 1,3,5 Triphenyl, (C) Cholesta-5,22-Dien-3-ol, and (D) Cholesta-3,5-Diene against the DNA gyrase A *S. typhi*

dien-3-ol and Cholesta-3,5-diene emerged as prominent sterol compounds belonging to the steroid group. These compounds were identified with high similarity indices of 80 and 79, respectively, further supporting their potential for therapeutic applications. The concentration of compounds in the extract can influence the size of the inhibition zone. Higher concentrations of bioactive compounds in the extract are likely to result in increased antibacterial activity, as reflected by larger inhibition zones. In this study, ciprofloxacin showed the highest inhibition due to its status as a pure compound, whereas the extract used was a crude extract with a mixture of various compounds. Binding energy is related to the intrinsic bioactive potential of individual compounds but is not affected by concentration. In the *in-silico* analysis, the binding energies of the three selected compounds were better than those of ciprofloxacin. Combining data on inhibition zones, compound concentrations, and binding energy provides a more comprehensive understanding of the potential of the compounds as antibiotic candidates.

Cholesta-5,22-dien-3-ol has been extensively studied for its diverse biological roles. It is a key precursor in the biosynthesis of vitamin D, an essential compound that modulates immune responses and supports overall physiological health (Cheseto *et al.* 2015). Furthermore, it exhibits anti-inflammatory properties likely linked to its role in cholesterol metabolism, as oxysterols derived from cholesterol are known to modulate inflammation and apoptosis (Schroepfer 2000). This connection suggests that Cholesta-5,22-dien-3-ol may share similar bioactivities, including immune modulation and anti-inflammatory effects. In addition to its role in inflammation, Cholesta-5,22-dien-3-ol has been reported as a significant antimicrobial component of essential oils (Değirmenci & Erkurt 2020). These findings, coupled with its identification in the *P. aurata* extract, underscore its potential as a candidate for further molecular docking studies to assess its binding efficiency and therapeutic viability against bacterial targets.

Cholesta-3,5-diene, also known as cholesterylene, exhibits a wide range of biological activities, particularly in wound healing and antibacterial applications. Al-Hassan *et al.* (2020) demonstrated that Cholesta-3,5-diene promotes tissue repair by recruiting neutrophils and fibroblasts, which are essential for wound regeneration. These findings suggest its therapeutic potential in dermatology, particularly in formulations targeting wound healing. Additionally, Cholesta-3,5-diene has been identified in catfish skin secretions, where it exhibits anti-cancer properties (Al-Hassan *et al.* 2022). This highlights a broader spectrum of biological activity, including potential cytotoxic effects against specific cell types. The antimicrobial properties of Cholesta-3,5-diene have been linked to its ability to disrupt microbial membranes, as reported by Akram *et al.* (2017). Furthermore, its presence in pyrolyzed tissues has been associated with cytotoxic and antibacterial effects, suggesting that its bioactivity extends beyond direct membrane interactions (Bae *et al.* 2018).

Cyclohexane, 1,3,5-triphenyl, identified in *P. aurata* extract, is known for its antibacterial and anti-inflammatory properties. Found in various plant extracts, such as *Vinca major*, it has been associated with notable therapeutic activities, including anti-inflammatory effects (Javaid *et al.* 2021). While its exact mechanisms remain unclear, its potential to disrupt microbial processes or inhibit bacterial enzymes aligns with the bioactivities observed in P. aurata extract. Alongside sterols like Cholesta-5,22-dien-3-ol and Cholesta-3,5-diene, this compound enhances the therapeutic profile of the extract, warranting further investigation into its role in multi-target antibacterial and anti-inflammatory effects.

The molecular docking studies focused on key bacterial proteins, highlighting their roles in pathogenicity and providing insights into the potential mechanisms of action for the bioactive compounds identified in *P. aurata* extracts. The target protein for *S. aureus* was Sortase-A (PDB ID: 1T2P) (Ragi *et al.* 2021). Sortases are extracellular transpeptidases in Gram-positive bacteria, responsible for sorting proteins into the cell wall compartments. This enzyme plays a critical role in cell wall assembly and bacterial pathogenicity. For *S. typhi*, the target protein was DNA gyrase A (PDB ID: 5ZTJ) (Nazeer *et al.* 2024). DNA gyrase A is a key target for antibiotics like quinolones, as it introduces negative supercoils into bacterial chromosomes, which are essential for replication and maintaining proper supercoiling levels (Levine *et al.* 1998).

Three major compounds from the methanol extract of *P. aurata* were selected for molecular docking analysis, with ciprofloxacin used as the positive control. These compounds were chosen based on their high % area in the sample and high Similarity Index, indicating their compliance for docking studies. Blind docking was initially performed with ciprofloxacin to identify the active site coordinates of the enzymes. For Sortase A, the active site residues were identified as Arg197, Asn114, Thr180, Ala104, and Trp194 (Song *et al.* 2022). For DNA gyrase A, the active site residues were Arg612, Lys550, and Gly613 (Nazeer *et al.* 2024). Once the best conformation was located within the enzyme's active site, molecular docking was performed for all ligands. The three selected compounds were Cyclohexane, 1,3,5-triphenyl, Cholesta-5,22-Dien-3-ol, and Cholesta-3,5-Diene, which are secondary metabolites classified under the steroid group. The structures of these compounds were optimized to ensure thermodynamic stability with low potential energy before docking.

All three compounds demonstrated better binding energies than ciprofloxacin, suggesting stronger interactions with the target protein. Ciprofloxacin formed hydrogen bonds with Asp112 and Arg197, while cyclohexane, 1,3,5 triphenyl interacted with Arg197 via pi-cation/pi-anion bonds, and Cholesta-5,22-Dien-3-ol and Cholesta-3,5-Diene formed van der Waals interactions. The interaction with Arg197 confirms that all three compounds are in the Sortase A binding pocket, where Arg197 serves as the active site residue (Song *et al.* 2022). Despite ciprofloxacin having more hydrogen bonds, the stronger binding energies of *P. aurata*

compounds can be attributed to extensive van der Waals interactions facilitated by the hydrophobic side chains in their structures. Previous studies have also reported the antibacterial activity of sterol mixtures containing Cholesta-5,22-Dien-3-ol and Cholesta-3,5-Diene (Reina *et al.* 2011), reinforcing their potential as antibacterial agents.

In the search for new drug candidates for typhoid fever treatment, several in silico studies have investigated ligands targeting *S. typhi* DNA gyrase A (Elseginy & Anwar 2022). Molecular docking was performed to evaluate the nonbonding and bonding interactions, as well as the binding energies of each ligand. All three compounds demonstrated better binding energies than ciprofloxacin, highlighting their strong interactions with the target protein. The binding interactions between the ligands and the DNA gyrase A active site involve key residues such as Arg612, Lys550, and Gly613 (Nazeer *et al.* 2024). All three ligands showed van der Waals interactions with Arg612, while Y3 also formed a hydrogen bond with Val685. Ciprofloxacin, as the positive control, interacted with Arg612 and Arg806 through hydrogen bonds. The 2D interaction diagrams confirm that all ligands are positioned within the active site of DNA gyrase A, supporting their potential as effective inhibitors.

This research underscores the potential of *P. aurata* as a source of bioactive compounds, such as Cholesta-5,22-dien-3-ol, Cholesta-3,5-diene, and Cyclohexane, 1,3,5-triphenyl, with significant antibacterial and anti-inflammatory properties. Due to their strong binding affinities against critical bacterial targets like Sortase-A and DNA gyrase-A, the compounds highlight their promise as next-generation antibiotics. In order to advance their applications, future studies should focus on scalable production methods, such as microbial biosynthesis or aquaculture, and develop formulations like nanoparticles or hydrogels to enhance bioavailability. Preclinical in vivo studies are essential for evaluating safety, pharmacokinetics, and efficacy, paving the way for potential clinical applications in treating infections and supporting wound healing.

In conclusion, This study demonstrates the promising potential of *P. aurata* as a source of bioactive compounds with significant antibacterial applications. The methanol extract exhibited notable efficacy against *S. aureus* and *S. typhi*, supported by the identification of key compounds such as Cholesta-5,22-dien-3-ol, Cholesta-3,5-diene, and Cyclohexane, 1,3,5-triphenyl through GC-MS analysis. These compounds showed strong binding affinities in molecular docking studies, surpassing ciprofloxacin in targeting critical bacterial proteins like Sortase-A and DNA gyrase A. The findings highlight *P. aurata* as a valuable candidate for developing novel antibiotic sources. Further research focusing on detailed bioactivity mechanisms and preclinical validation is essential to translate these findings into practical therapeutic applications.

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