Steroidal and Potential Anti-inflammatory Properties of *Carteriospongia* sp from the Southeast Sulawesi Sea Coast

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Abstract

Marine sponges are an abundant yet underutilized resource in Southeast Sulawesi, Indonesia, despite their ecological roles and pharmacological potential. Steroidal metabolites are dominant in many sponge species and have been widely recognized for their strong anti-inflammatory activity. However, the chemical composition and biological potential of Carteriospongia sp from Indonesian waters remain unexplored. Carteriospongia sp was extracted using the Soxhlet method, and the chemical profile was analyzed by Gas Chromatography–Mass Spectrophotometry (GC-MS). Anti-inflammatory activity was evaluated in vitro using the Bovine Serum Albumin (BSA) protein denaturation assay, with methylprednisolone as the positive control. Molecular docking studies were performed to assess the interactions between steroidal compounds and BSA protein. GC-MS analysis identified eight steroidal compounds such as 2-methylenecholestan-3-ol (S1), stigmasterol (S2), 4,4-dimethyl-cholesta-8,24-dien-3-ol (S3), 8,14-seco-3,19-epoxyandrostane-8,14-dione, 17-acetoxy-3ß-methoxy-4,4-dimethyl (S4), cholic acid ethyl ester (S5), (3ß,5Z,7E)-9,10-secocholesta-5,7,10(19)-triene-3,24,25-triol (S6), stigmastan-3-en-6-ol (S7), ß-sitosterol (S8). The extract demonstrated concentration-dependent inhibition of protein denaturation, achieving 84.81% inhibition at 128 mg.L^{-1}. The IC_{50} value was 3.71 mg.L^{-1}, comparable to methylprednisolone (IC_{50} 4.45 mg.L^{-1}). Molecular docking revealed that compound S1 and S4 exhibited lower binding energies than other steroids, suggesting stronger stabilization of BSA protein. The findings demonstrate that Carteriospongia sp extract possesses significant anti-inflammatory potential, comparable to methylprednisolone. This study provides novel insights into the bioactive potential of Indonesian marine sponges and emphasizes the importance of further pharmacological and molecular studies to develop sponge-derived steroidal compounds as alternative or complementary anti-inflammatory therapeutics.

Keywords: *Carteriospongia sp. Steroid, protein denaturation, anti-inflammatory, Southeast Sulawesi*

Introduction

Indonesia has 17,504 islands and 95,181 km of coastline with the availability of potential natural resources both in coastal areas and marine waters. Southeast Sulawesi Waters on Sulawesi Island has large fishery resources, tourism potential and biodiversity that has not been optimally utilized, mostly from coral reef ecosystems (Tuwo *et al*., 2021). In coral reef ecosystems, sponges have a complex ecological role (Coppock *et al*., 2024; Rovellini *et al*., 2024). In general, sponge populations are not managed effectively, and are referred to as 'neglected groups' (Sayori *et al*., 2022), despite their important role due to their bioactive compounds, as research, management, and conservation efforts regarding their diversity and pharmacological potential remain limited.

As many as 38 families and 100 species of sponges are recorded from Southeast Sulawesi waters (Johan *et al*., 2024). To date, only a few of them have been explored, including *Calispongia* sp (Fristiohady *et al*., 2024), *Xestospongia* sp (Sadarun *et al*., 2022; Sudayasa *et al*., 2020), *Aaptos* sp (Fristiohady *et al*., 2020), *Petrosia* sp (Fristiohady *et al*., 2021), *Clathria* sp (Sahidin *et al*., 2020) and *Melophlus sarasinorum* (Wahyuni *et al*., 2019). Pharmacological studies of some of these sponges have also been studied and are able to provide activity as antibacterial (Kumar, 2022), anti-inflammatory (Fristiohady *et al*., 2024; Magri *et al*., 2023), antioxidant (Martignago *et al*., 2023), anticancer (Bashari *et al*., 2019) and antidiabetic (Pathak *et al*., 2022). These pharmacological properties are linked to the chemical diversity of sponges, as further studies have identified various bioactive metabolites.

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The identification results showed that the sponge contained metabolite compounds such as N-Docosanoyl-4-sphingenine, (4Z,9Z)-Dehydrosiphonochalynol, 4E,16E-Eicosadiene-1,19-diyne-3S,18S-diol, α-Spinasterol, Fucosterol, Isofucosterol, 3-Hexadecyloxy-1,2-propanediol, 3β-(Acetoxymethyl-)-A-nor-5α-cholestane, Ergosterol, 3β-(Hydroxymethyl-)-A-nor-5α-cholest-15-ene, 3β-(Hydroxymethyl-)-A-nor-5α-cholestane, Ornithine, α-Ionone, Gentiatibetine, Tryptophan, 8,9-Epoxy-3-hydroxy-4,4-dimethyl-30-nor-8,9-secocholesta-9(11),8(14),24-trien-23-one, Laminine, Oxyphyllenodiol A, Xestosaprol C methylacetal, Mutafuran H, Fucosterol, Isofucosterol, Saringosterol, Xestosterol, Pulchrasterol, Pyrophaeophorbide and Spinasterone (Sahidin *et al*., 2020). Among these compounds, steroid derivatives are the most dominant, which is noteworthy since this class of metabolites has been widely recognized for its strong anti-inflammatory properties.

Steroids derived from marine sponges have shown promising anti-inflammatory properties. Several steroid compounds identified from marine sponges have been reported to exhibit well-defined anti-inflammatory mechanisms. Fucosterol is known to suppress the activation of the NF-κB and MAPK (ERK, JNK, p38) pathways, thereby reducing the expression of iNOS and COX-2, while simultaneously enhancing antioxidant responses through the activation of Nrf2/HO-1 (Liu *et al*., 2025). Spinasterol shows a similar mechanism by inhibiting MAPK phosphorylation and the NF-κB pathway, leading to decreased production of pro-inflammatory mediators such as TNF-α, IL-6, COX-2, and 5-LOX, and has also been reported to increase Nrf2/HO-1 expression in macrophages (Lee *et al*., 2012). Meanwhile, ergosterol and its derivatives (e.g., ergosterol peroxide) act by suppressing the TLR4 NF-κB signaling pathway, as well as inhibiting the transcription factor C/EBPβ and MAPK phosphorylation, making them relevant for anti-neuroinflammatory activity (Rangsinth *et al*., 2023). Another steroid, 24(S)-saringosterol, works through a different mechanism, namely as an LXR (LXRα/β) agonist. LXR activation is known to repress the transcription of pro-inflammatory genes through trans-repression of NF-κB/MyD88, and has been associated with the improvement of inflammatory phenotypes, particularly in neurodegenerative disease models. Taken together, these findings highlight marine sponge derived steroids as promising anti-inflammatory agents with diverse molecular targets.

Several descriptions indicate that sponges possess many interesting and important metabolites and properties, particularly as anti-inflammatory agents. However, their chemical constituents and properties are still underexplored compared to their population in Southeast Sulawesi. Therefore, to reveal more in-depth potential, chemical and pharmaceutical studies of other species are essential. In this context, chemical and pharmaceutical exploration of several sponge species remains limited, especially the *Carteriospongia* sp. from Southeast Sulawesi waters, which is completely unknown. This study focuses on the steroid content of this sponge and evaluates its anti-inflammatory activity through the inhibition of protein denaturation.

Materials and Methods

Carteriospongia sp samples were obtained from the sea coast of Waworaha village, Soropia sub-district, Konawe district, Southeast Sulawesi. Extraction was carried out using the Soxhletation technique, in which 50 g of dried sponge powder was extracted with 500 mL of 96% ethanol at a controlled temperature of 60–70°C for 8 h until the solvent in the siphon tube became clear (Varijakzhan *et al*., 2021). The obtained extract was concentrated under reduced pressure using a rotary evaporator at 40°C to remove excess solvent and then stored at 10°C in a dark container before further testing. Qualitative identification of steroid compounds was carried out by the colorimetric method, specifically the Liebermann–Burchard test, where the formation of a blue-green color indicates the presence of steroids (Godlewska *et al*., 2022).

Structural analysis of steroid compounds from the extract was carried out by Gas Chromatography Mass Spectrophotometry (GCMS) according to established procedures (Yodha *et al*., 2023). The GC-MS analysis was performed using a capillary column (HP-5MS, 30 m × 0.25 mm × 0.25 μm). Helium was used as the carrier gas at a constant flow rate of 1 $mL.min^{-1}$. The oven temperature was programmed as follows: initial temperature at 60°C (held for 2 min), increased to 280°C at a rate of $10°C.min^{-1}$, and then held for 10 min. The injector temperature was set at 250°C with a split ratio of 1:20, and the detector (MS) was operated in electron ionization (EI) mode at 70 eV with a scanning range of $m.z^{-1}$ 50–600. The structure of steroid compounds was determined based on the similarity index of molecular ion fragmentation patterns compared to the NIST library, while the relative content was calculated based on the peak area of the resulting chromatogram.

The extracts were evaluated for their anti-inflammatory activity in vitro using the protein denaturation inhibition technique against Bovine Serum Albumin (BSA). The assay refers to a previous study with slight modifications (Yodha *et al*., 2024). A 1% (w/v) BSA solution was freshly prepared in phosphate buffer saline (PBS, pH 6.4). The test

samples were dissolved in ethanol and further diluted with buffer to obtain concentrations ranging from 2 to 128 µg.mL^{-1}, ensuring that the final solvent concentration did not exceed 1% (v/v). Methylprednisolone was used as the positive control and prepared at the same concentration range. or each reaction, 100 µL of BSA solution was mixed with 100 µL of the test sample or standard in microtubes. The mixtures were incubated at 37°C for 20 min and subsequently heated at 70°C for 5 min to induce denaturation. After cooling to room temperature, turbidity was measured spectrophotometrically at 660 nm against buffer as the blank. The inhibitory activity is expressed according to equation 1 below:

$$\text{Inhibitory activity (\%)} = \frac{AC-AS}{AC} \times 100 \quad (1)$$

Note: AC= absorbance of the heat-denatured BSA without sample, and AS= absorbance in the presence of extract or standard. The IC_{50} value of sample was determined by: (a) Inhibitory activity (y) plotted against concentration (x) at seven points (2, 4, 8, 16, 32, 64 and 128 mg.L^{-1}), (b) regression line equation is determined (y= ax+b), and (c) sample concentration (x) is calculated by substituting y=50 in the regression equation (b)

The molecular interactions between steroid compounds derived from *Carteriospongia* sp and BSA protein were examined through docking studies. The BSA protein, identified by PDB ID 6QS9 (Castagna *et al*., 2019), was sourced from the Protein Data Bank. Concurrently, the three-dimensional structures of the steroid compounds were obtained from the PubChem database and subjected to geometry optimization using the semi-empirical AM1 method in ChemDraw 15.0. The preparation of the protein and compound structures adhered to the protocols outlined in our prior research (Arfan *et al*., 2024).

The molecular docking procedure was executed using AutoDock Vina, which utilized a cubic grid with dimensions of 25 x 25 x 25 Å and center coordinates of -78.807, 4.473, and 36.53 for the x, y, and z axes, respectively. Default parameters were applied during the docking process, including 9 conformational searches and an exhaustiveness setting of 32. To validate the docking method, the native ligand (JGE) was re-docked onto BSA. This docking method is deemed reliable if the re-docked conformation of the JGE demonstrates a Root Mean Square Deviation (RMSD) value of ≤ 2Å when compared to its crystallographic conformation. The assessment of molecular docking focused on selecting ligand configurations that exhibited the most favorable binding free energy (ΔG). The resulting complexes of compounds and proteins were visualized using Discovery Studio Visualizer software.

Result and Discussion

The extraction process of *Carteriospongia* sp (120 g) produced 2.7 g of extract (2.5%). Qualitative identification showed a positive reaction showing a green color change. The formation of complexes between Liebermann reagent and the functional groups of steroid compounds in the extract causes this color change. Further quantitative analysis of *Carteriospongia* sp extract by GCMS showed the presence of eight major peaks with retention times (RT) between 23.9 and 29.5 min (Figure 1). The major peaks indicate the presence of compounds with large molecular weights, which are generally steroids. The interpretation of each peak is presented in Table 1, which provides information on retention time, peak area, molecular mass, fragment ions, molecular formula, and the corresponding compound identification.

The structure of steroid compounds from GCMS analysis is determined based on the fragmentation of compound ions in mass spectrometry which involves the breakdown of steroid molecules into smaller ionic fragments. The fragments are the result of breaking the bond between carbon (C-C), breaking the cyclohexane ring and releasing functional groups such as hydroxyl (-OH), carbonyl (C=O) and methyl (CH_3) (McDonald *et al*., 2011; Yan *et al*., 2014). Steroid compounds generally exhibit characteristic fragmentation patterns resulting from cleavage of the tetracyclic *cyclopentanoperhydrophenanthrene* skeleton. Common fragment ions appear at m.z^{-1} 55, 69, 81, 95, and 121, which originate from the cleavage of aliphatic side chains. In addition, ions at m.z^{-1} 255–315 usually represent stable fragments of the steroid rings, while higher ions such as m.z^{-1} 381–397 indicate the presence of almost the entire steroid backbone with minor losses of side chains or hydroxyl groups. Each pattern formed is used to identify the shape of the molecule through data comparison with reference spectra or mass spectrum databases.

Based on this, it is known that the 8 steroids contained are 2-methylenecholestan-3-ol (S1), stigmasterol (S2), 4,4-dimethyl-cholesta-8,24-dien-3-ol (S3), 8,14-seco-3,19-epoxyandrostane-8,14-dione, 17-acetoxy-3ß-methoxy-4,4-dimethyl (S4), cholic acid ethyl ester (S5), (3ß,5Z,7E)-9,10-secocholesta-5,7,10(19)-triene-3,24,25-triol (S6), stigmastan-3-en-6-ol (S7), ß-sitosterol (S8). The structures of these steroid compounds are shown in Figure 2.

The presence of steroid compounds in *Carteriospongia* sp is believed to play a significant role in modulating its anti-inflammatory activity. Steroids are well known for their ability to inhibit the production of pro-inflammatory mediators and

stabilize cellular components during inflammatory responses. This potential is reflected in the experimental findings (Figure 3), particularly in the inhibition of BSA protein denaturation, where the extract demonstrated a dose-dependent activity comparable to the reference drug methyl-prednisolone.

Figure 3A shows the percentage of inflammatory activity inhibition by the *Carteriospongia* sp extract at various concentrations (2–128 mg.L^{-1}) compared with the positive control methylprednisolone. The results indicate that both *Carteriospongia* sp extract and methylprednisolone exhibited increased inhibition with rising concentrations.

Table 1. GCMS analysis of *Carteriospongia* sp extract

No	RT (min)	Area Count	Mass (Da)	Fragment mass ion (m.z^{-1})	Formula	Compounds	Structure
1	23.934	10101700.81	400.6801	69; 81; 95; 121; 315	$C_{28}H_{48}O$	2-methylenecholestan-3-ol	S1
2	25.103	7539099.00	412.6908	55; 69; 83; 159; 255	$C_{29}H_{48}O$	stigmasterol	S2
3	25.287	7083115.84	412.6908	43; 55; 69; 109; 259	$C_{29}H_{48}O$	4,4-dimethyl-cholesta-8,24-dien-3-ol	S3
4	25.447	17142300.97	420.5390	55; 69; 83; 96; 111; 177; 265	$C_{24}H_{36}O_6$	8,14-seco-3,19-epoxyandrostane-8,14-dione, 17-acetoxy-3ß-methoxy-4,4-dimethyl	S4
5	25.858	7539099.00	436.6331	41; 55; 81; 271; 372	$C_{26}H_{44}O_5$	cholic acid ethyl ester	S5
6	26.994	10060640.78	416.6365	55; 118; 136; 158; 207; 383	$C_{27}H_{44}O_3$	(3ß,5Z,7E)-9,10-secocholesta-5,7,10(19)-triene-3,24,25-triol	S6
7	29.429	16394367.46	414.7067	43; 95; 149; 381; 397	$C_{29}H_{50}O$	stigmastan-3-en-6-ol	S7
8	29.552	8366675.54	414.7067	43; 55; 81; 95; 107	$C_{29}H_{50}O$	ß-sitosterol	S8

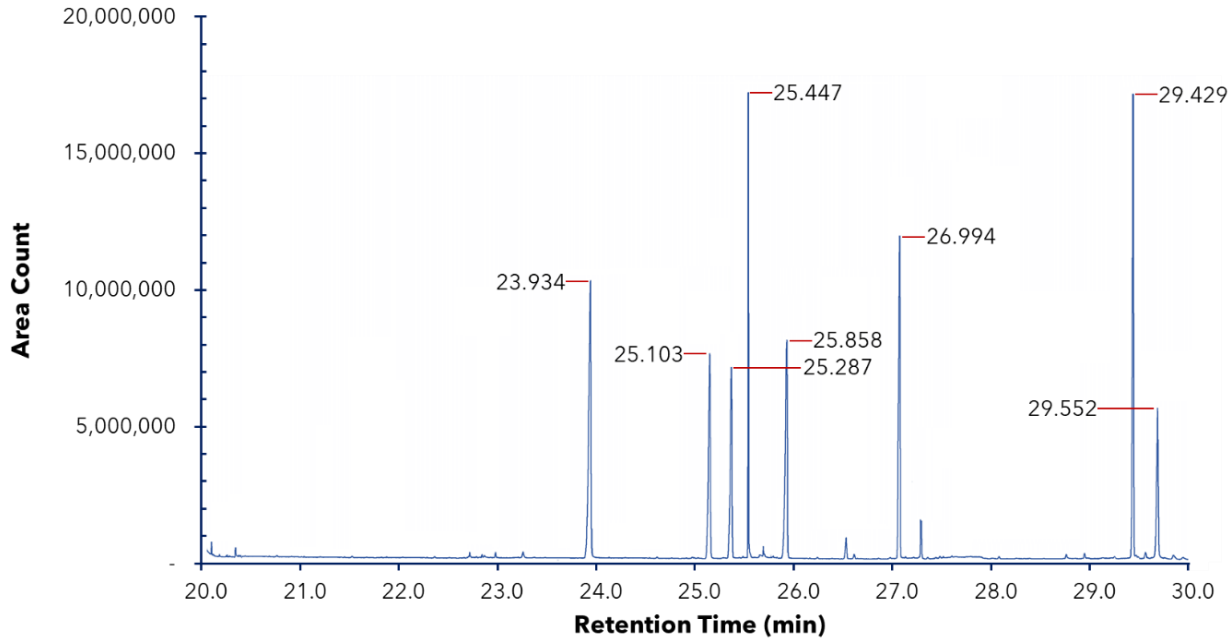

Figure 1. GC-MS chromatogram of *Carteriospongia* sp extract

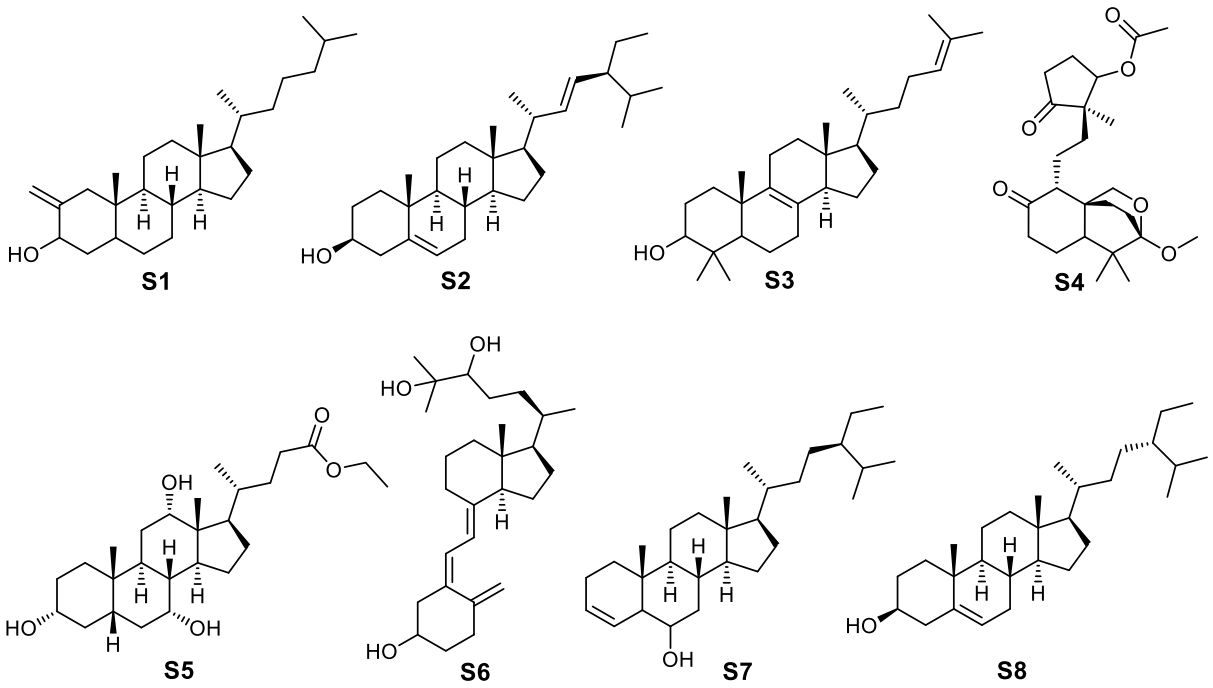

Figure 2. Structure of steroidal from *Carteriospongia* sp extract

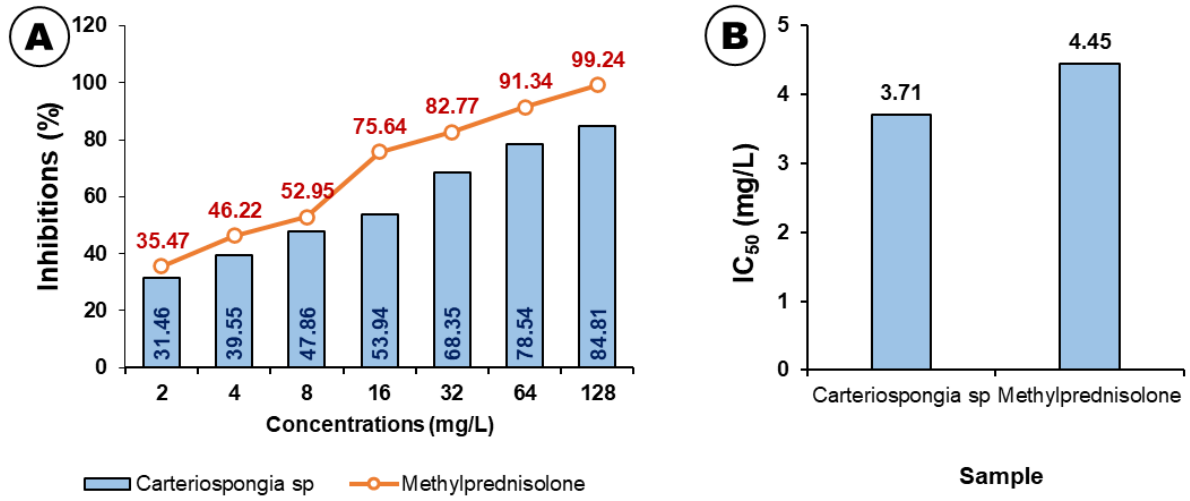

Figure 3. Anti-inflammatory activity of *Carteriospongia* sp extract compared with methylprednisolone

At the highest concentration (128 mg.L^{-1}), *Carteriospongia* sp extract produced an inhibition of 84.81%, while methylprednisolone reached 99.24%. Figure 3B illustrates the IC_{50} values, which represent the concentration required to inhibit 50% of the inflammatory response. The IC_{50} value of *Carteriospongia* sp extract was 3.71 mg.L^{-1}, lower than that of methylprednisolone (4.45 mg.L^{-1}). This finding indicates that *Carteriospongia* sp extract possesses anti-inflammatory potential comparable to, and even slightly stronger than, methylprednisolone based on the IC_{50} parameter. These results strengthen the potential of *Carteriospongia* sp as a natural bioactive source with promising anti-inflammatory activity, making it a potential candidate for development as an alternative or complementary agent to synthetic anti-inflammatory drugs.

The results of the activity assay further underscore the pharmacological relevance of the extract and highlight the importance of conducting advanced studies on the molecular interactions of its

bioactive constituents. In this analysis, we evaluated the interactions of several steroid compounds from *Carteriospongia* sp extract with BSA protein using molecular docking methods to assess the ability of these compounds to stabilize BSA protein and correlate it with potential anti-inflammatory activity. The docking procedure was validated with an RMSD parameter of 1.4Å (Figure 4A). The molecular interactions of methylprednisolone with BSA involve hydrogen bonds with Ser191, Arg194, and Glu291, as well as hydrophobic interactions with Tyr149, Trp231, Arg217, Leu237, His241, and Ala290 (Figure 5). These interactions are critical for stabilizing the drug-protein complex (Figure 4B).

The docking results of steroid compounds from the extract of *Carteriospongia* sp against bovine serum albumin (BSA) protein reveal significant insights into their molecular mechanisms and potential anti-inflammatory activities. Compounds S1 and S4 demonstrate binding energies of -8.9 kcal.mol^{-1} and -9.0 kcal.mol^{-1}, respectively, closely matching the binding energy of the known anti-inflammatory agent methylprednisolone (MP) (-9.1 kcal.mol^{-1}) (Figure 5). This similarity in binding affinity suggests that these compounds may exhibit comparable anti-inflammatory effects. Compounds S3, S6, S7, and S8 show more positive binding energies, ranging from -8.0 to -8.2 kcal.mol^{-1}, indicating moderate interactions with BSA. In contrast, compounds S2 and S5 exhibit significantly more positive binding energies of -7.5 and -7.7 kcal.mol^{-1}, respectively, which may correlate with their lower ability to stabilize the BSA protein.

In this study, bovine serum albumin (BSA) was used as the target protein in molecular docking to evaluate interactions with potential anti-inflammatory compounds. BSA denaturation is a commonly used in vitro method for screening anti-inflammatory activity, based on the principle that many anti-inflammatory agents function by stabilizing protein structures against heat- or chemical-induced denaturation (Williams *et al*., 2008). Accordingly, a compound's strong binding affinity (*i.e.,* more negative binding energy) to BSA may indicate its potential to prevent protein unfolding and aggregation, thereby reflecting possible anti-inflammatory effects in vivo (Williams *et al*., 2008; Rout *et al*., 2023).

The binding energy values derived from molecular docking simulations represent the theoretical affinity between the ligand and the target protein. More negative binding energy values indicate stronger and thermodynamically more favorable interactions (Du *et al*., 2016; Alotaiq and Dermawan, 2025). In the context of BSA denaturation assays, such enhanced binding affinities may correlate with an increased ability of the compound to stabilize the protein structure and prevent thermal or chemical-induced denaturation (Celej *et al*., 2003).

Molecular interaction of Compound S1, despite lacking hydrogen bonds, forms ten hydrophobic interactions with BSA residues (Tyr149, Trp213, Leu218, Phe222, Leu237, His241, Arg256, Leu259, Ile263, and Ala290), suggesting that hydrophobic forces play a crucial role in its binding affinity (Figure 6A). The predominance of hydrophobic interactions may influence the compound's stability and its potential to modulate protein function, contributing to its anti-inflammatory properties. On the other hand, Compound S4 establishes four hydrogen bonds with Ser191, Arg217, Lys221, and His241, and engages in hydrophobic interactions with Arg194, Trp213, Leu218, His287, and Ala290 (Figure 6B). These two compounds exhibit molecular interactions with BSA protein that are analogous to those observed with methylprednisolone, suggesting a potential anti-inflammatory activity of comparable efficacy.

The molecular docking results for the other steroid compounds indicate that Compound 2 forms a single hydrogen bond with the Lys187 residue, whereas Compounds 3, 7, and 8 do not form any hydrogen bonds. Conversely, Compound 5 establishes five hydrogen bonds with the residues Tyr156, Lys187, Arg194, Arg256, and Ala290, and Compound 6 forms three hydrogen bonds with the residues Arg256, Ser286, and Glu291 (Figure 7). Further analysis reveals that all these compounds consistently form hydrophobic interactions with the residues Leu259, Arg256, Ile263, Trp213, Leu218, Lys221, Phe222, Arg217, His241, Ala260, and Ala290 at the BSA protein binding site. These findings collectively suggest that steroid compounds derived from *Carteriospongia* sp extract possess the capability to interact with and stabilize BSA protein through the formation of hydrogen bonds and hydrophobic interactions with residues at the BSA protein binding site.

Methylprednisolone is a steroid class drug that exerts anti-inflammatory effects through various mechanisms. In Alzheimer's disease, methyl-prednisolone exhibits neuroprotective effects by ameliorating cognitive impairment, suppressing microglial activation, and improving synapse function (Sun *et al*., 2023). In severe acute pancreatitis, methylprednisolone reduced inflammatory cytokines, ameliorated pancreatic and lung injury, and downregulated NF-κB and NLRP3 inflammasome activation (Liu *et al*., 2020). In addition, methylprednisolone has been shown to suppress LPS-induced microglial activation by reducing NF-κB signaling and MAPK phosphorylation. In tuberculosis,

methylprednisolone promotes mycobacterial proliferation in macrophages by suppressing ROS production and IL-6 secretion through downregulation of NF-κB and upregulation of DUSP1 (Li *et al*., 2023).

Like methylprednisolone, steroid compounds from *Carteriospongia* sp extract also have a major role as anti-inflammatory. Research shows that stigmasterol can reduce inflammatory responses by reducing the release of inflammatory mediators, inhibiting enzymes such as iNOS and COX-2, and promoting the production of anti-inflammatory cytokines (Bakrim *et al*., 2022). Moreover, 2-methylenecholestan-3-ol present in Bidens tripartita has demonstrated potential in reducing levels of proinflammatory cytokines interleukin 8 (CXCL8) and interleukin 6 (IL-6) (Antoniak *et al*., 2023). In addition, another steroid, β-Sitosterol, exhibits anti-inflammatory effects by suppressing inflammatory responses through the NF-κB and p38 MAPK signaling pathways (Zhou *et al*., 2020).

These findings underscore the importance of investigating steroidal metabolites derived from marine sponges such as *Carteriospongia* sp, as their mechanisms of action parallel those of clinically used corticosteroids like methyl-prednisolone. By modulating key inflammatory pathways, including NF-κB, MAPK, iNOS, and COX-2, these compounds demonstrate significant potential in attenuating excessive inflammatory responses. Furthermore, elucidating the molecular interactions of these bioactive steroids through advanced approaches such as molecular docking and in vivo validation will be critical to determine their pharmacological relevance and

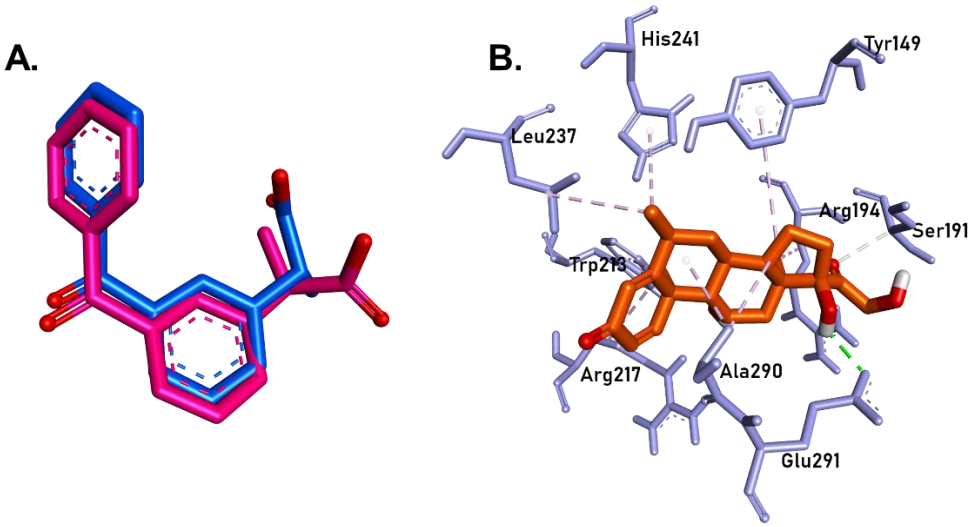

Figure 4. (A) Superimposed crystal conformation of JGE (blue) and its validated conformation (pink). (B) Molecular interactions of methylprednisolone with BSA protein.

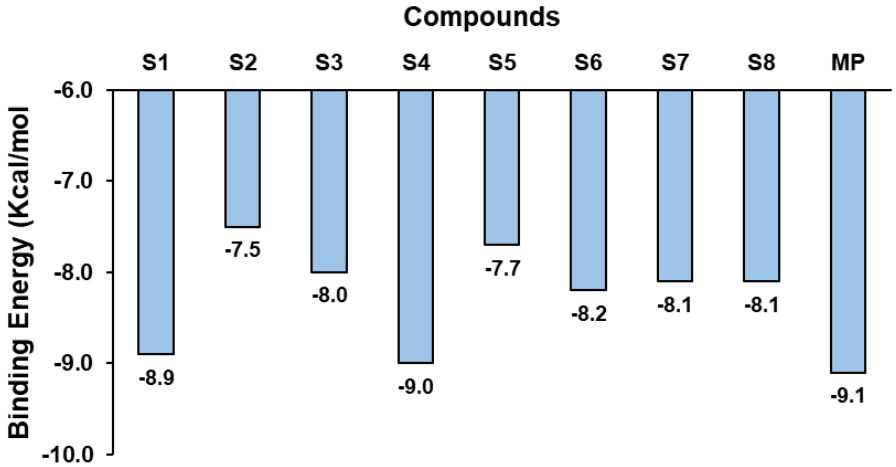

Figure 5. Binding energy of steroid compounds from the extract of *Carteriospongia* sp against bovine serum albumin (BSA) protein

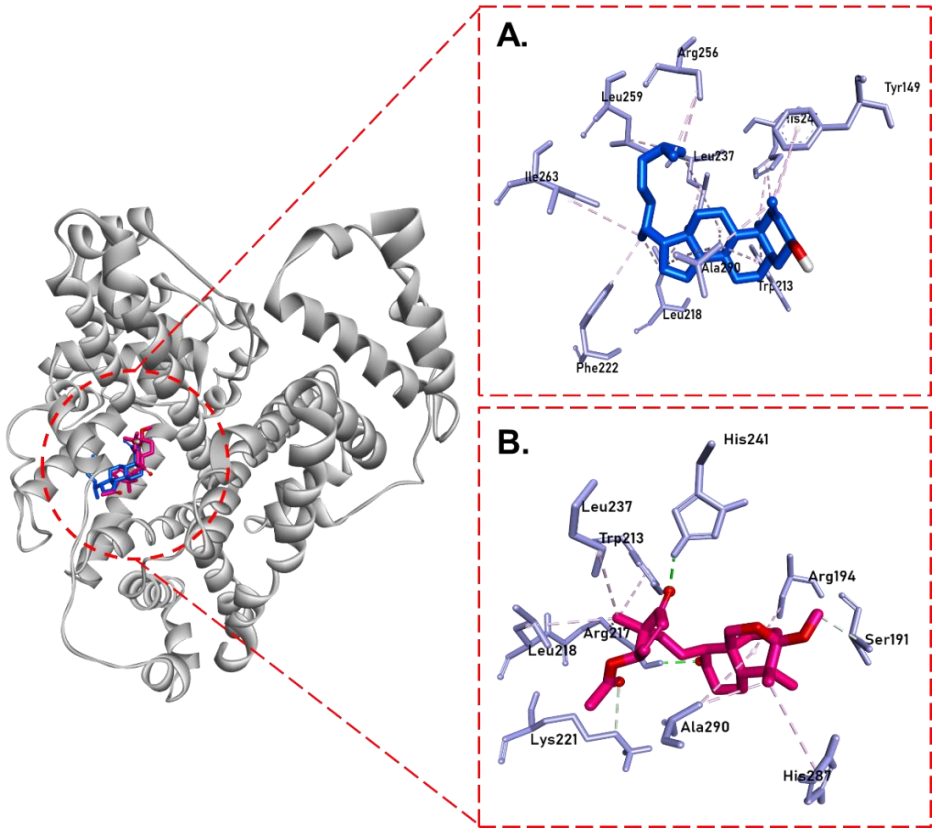

Figure 6. Molecular interactions of (A) Compound S1 and (B) Compound S4 from *Carteriospongia* sp extract with BSA protein.

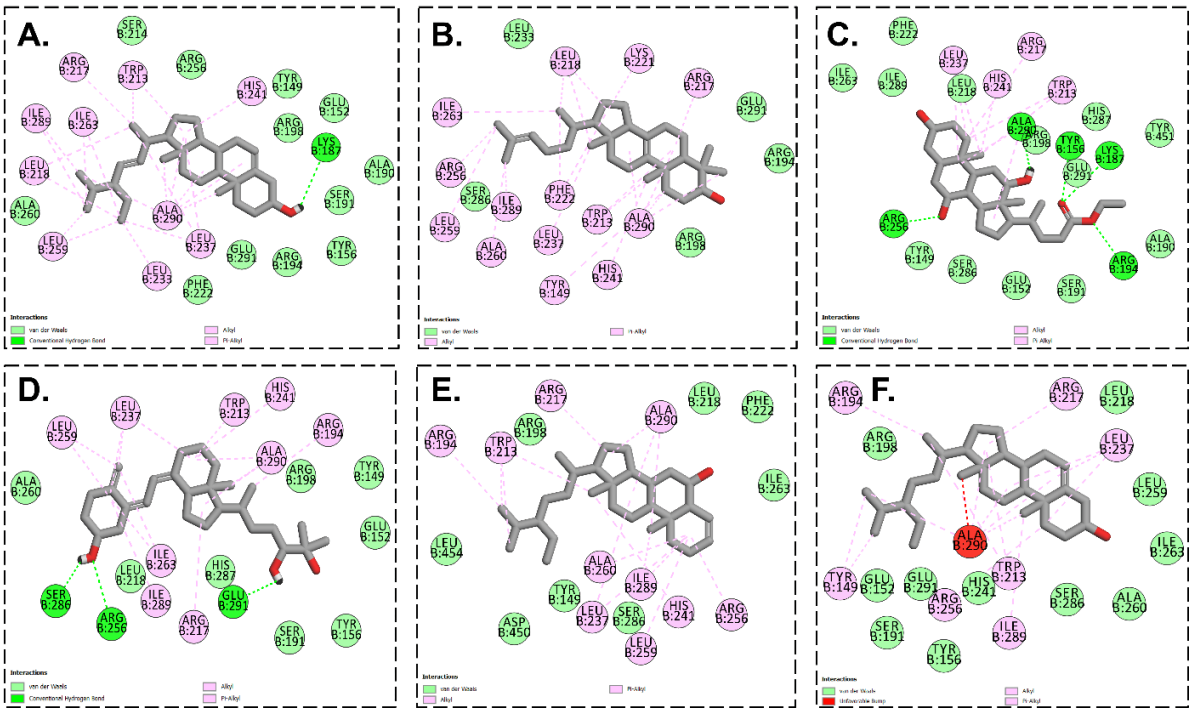

Figure 7. Molecular interactions of (A) Compound S2, (B) Compound S3, (C) Compound S5, (D) Compound S6, (E) Compound S7, and (F) Compound S8 from *Carteriospongia* sp extract with BSA protein.

safety profile. Such insights not only provide a deeper understanding of their anti-inflammatory mechanisms but also pave the way for the rational development of novel, natural-product-based therapeutics that could serve as alternatives or complementary agents to existing synthetic anti-inflammatory drugs.

Conclusion

Carteriospongia sp extract contains eight steroidal compounds such as 2-methylenecholestan-3-ol (S1), stigmasterol (S2), 4,4-dimethyl-cholesta-8,24-dien-3-ol (S3), 8,14-seco-3,19-epoxyan drostane-8,14-dione, 17-acetoxy-3ß-methoxy-4,4-dimethyl (S4), cholic acid ethyl ester (S5), (3ß,5Z,7E)-9,10-secocholesta-5,7,10(19)-triene-3,24,25-triol (S6), stigmastan-3-en-6-ol (S7), ß-sitosterol (S8). The ability of the extract, which is equivalent to methylprednisolone in inhibiting protein denaturation. Additionally, 2-methylenecholestan-3-ol and 8,14-seco-3,19-epoxyandrostane-8,14-dione exhibit lower binding energies compared to other steroid compounds, suggesting that these two compounds may stabilize BSA protein more effectively, making them potential candidates for development as anti-inflammatory agents. This study provides new insights into the bioactive potential of marine sponges from Indonesian waters, particularly *Carteriospongia* sp, which remains underexplored compared to other marine resources. The findings not only expand the current understanding of steroid metabolites in sponges but also emphasize the relevance of Indonesian marine biodiversity as a valuable source of novel pharmacological agents. Future studies should include in vitro and in vivo validation of these steroid compounds, as well as detailed molecular investigations to confirm their mechanisms of action. Such research would strengthen the foundation for developing marine sponge-derived natural products as alternative or complementary anti-inflammatory therapeutics.

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