Pharmacological activity of compounds isolated from methanolic extract marine sponge *xestospongia* sp. against *escherichia coli* and *staphylococcus aureus*

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Abstract. Few previous studies show pharmacological potencies of Xestospongia sp. and have not been explored further about its antibacterial activity. Thus, this study aims to isolate and identify the isolates from Xestospongia sp. and testing their antibacterial activity. Xestospongia Sp. was macerated with methanol then isolated and was purified by using vacuum liquid chromatography (VLC), radial chromatography (RC) and thin-layer chromatography (TLC). Isolated compounds then analyzed, identified, and determined their structures using 1H-NMR Spectrophotometer and by comparing data to references and ChemDraw 8.0. Compounds obtained with various concentrations (1000; 500; 100 μg/mL) then tested against Staphylococcus aureus ATCC 25923 and Escherichia coli ATCC 35218 using agar well-diffuse method. According to the study, two compounds isolated which were Saringosterol (isolate X1) and predicted-compound halenaquinone (isolate X2). The antibacterial test showed that isolate X1 was unable to inhibit bacteria’s’ growth at each concentration, and isolate X2 showed antibacterial activity against S. aureus ATCC 25923 and E. coli ATCC 35218 at concentration 1000 and 500 μg/mL. In conclusion, 2 compounds successfully isolated from methanolic extract of Xestospongia sp. which were Saringosterol and predicted-compound halenaquinone, and only predicted-compound halenaquinone showed antibacterial activity at concentration 1000 and 500 μg/mL.

1. Introduction

The high incidence of infectious diseases caused by bacteria is a problem that still occurs in Indonesia. The tropical climate, warm temperature, high humidity, as well as lack of awareness and individual knowledge to maintain personal hygiene are factors in the occurrence of disease [1]. The widespread availability of antibiotics among people tends to make the use of antibiotics irrational, such as incomplete use of antibiotics and inappropriate use of antibiotics [2]. A significant increase in the irrational use of antibiotics leads to bacterial resistance against antibiotic. Antibiotic resistance is one of the biggest threats to global health which results to longer hospital stays, higher medical costs and increased mortality [1].

Finding novel antibacterial agents is a solution to overcome the threat. Marine sponges are one of the group marine biota found in vast Indonesian seas with a total of 850 species and potentially
produce secondary metabolites with bioactive properties [3]. *Xestospongia* sp. belongs to *Demospongia* class, is reported to have broad pharmacological aspect studies such as anti-inflammatory agent, antioxidant, immunomodulator, cytotoxicity activity, antimicrobial, insecticide, HIV-protease inhibitor activity, anti-plasmodial activity, and anti-hyperlipidemic [4,5].

*Xestospongia* sp. provides the opportunity for discovering novel bioactive compounds as antibacterial [6]. Antibacterial activity of *Xestospongia* sp. reported in the previous study [7], compounds such as 8-hydroxyrenierin-2 and strongylodiol A have activity against *P. aeruginosa* and *M. intracellulare*. Thus, this study aims to investigate the antibacterial activity of isolates from methanolic extract of *Xestospongia* sp.

2. Material and Methods

2.1. Sponge Collection and Preparation

Marine sponge used in the study is *Xestospongia* sp. The sponge was obtained in reef slope 70º and 10 m depth with SCUBA in Bintang Samudra Marine Edu-Park, Soropia, Konawe, Southeast Sulawesi. Collected sponge was washed in running water and air-dried. Continued by dried sponge was cut into pieces then powdered.

2.2. Extraction

3.7 kg of dried powdered of marine sponge was macerated for 3 x 24 hour in jar by using 15 L methanol (MeOH). Filtrate obtained then concentrated by using vacuum rotary evaporator and yielded 173 g concentrated extract (4.67%).

2.3. Isolation, Purification, and Identification of Isolates

Isolation and purification of chemical constituent of methanolic extract *Xestospongia* sp. conducted by Thin Layer Chromatography (TLC), Vacuum Liquid Chromatography (VLC) and Radial Chromatography (RC). Profile data of isolates measured and collected from TLC, and NMR. Data obtained were compared to literature.

Initial test was using TLC of methanolic extract of *Xestospongia* sp. by using mixture n-hexane and ethyl acetate (EtOAC) with increased polarity (9:1; 8:2; and 7:3) for mobile phase (eluent).

25 g of extract was fractioned by using VLC with 13.5 cm of diameter and 4 cm thickness, n-hexane : EtOAC for eluent with increased polarity (9:1; 8:2; and 7:3) obtained 15 fractions. Fractions then observed by using TLC with n-hexane : EtOAC (9:1). Fractions with similar TLC profile were combined and obtained 3 main fractions which were fraction A (1.2 g), fraction B (3.55 g) and fraction C (0.79 g). Fraction C then re-fractioned by RC with n-hexane:EtOAC (7:3) eluent then obtained isolate 1 (X1). Fraction A and Fraction B were combined then re-refraction by VLC with as n-hexane:EtOAC (8:2) as eluent then obtained isolate 2 (X2). Isolates obtained were then interpreted and compared by references.

2.4. Antibacterial Activity Test of Isolates

Agar well-diffusion method was chosen for antibacterial activity test of isolates. DMSO and chloramphenicol were used as normal control and positive control respectively. 250 µg/mL *Staphylococcus aureus* ATCC 25923 inocula (0.5 McFarland) were plated onto Nutrient Agar (NA). Wells were punched in the plate by using sterile stainless steel borer. The wells were filled by 30 µl isolates with various concentrations (1000 µg/mL, 500 µg/mL, and 100 µg/mL); DMSO 30 µg/mL; and chloramphenicol 100 µg/mL. Same procedure was used for testing susceptibility of isolates against *Escherichia coli* ATCC 35218 inocula. Plates then put in refrigerator at 10°C for 30 minutes and then continued by incubating at 37°C for 24 hours in the incubator. The diameters of the inhibitory zone were measured in millimeters (mm).
3. Results and Discussion

3.1. Isolation and Purification of Isolates
Two (2) chemical isolate were successfully isolated and identified from methanolic extract of marine sponge Xestospongia sp. Structures of these isolates were determined by spectra of $^1$H-NMR and by comparison data to references.

3.1.1. Isolate X1
Spectra of $^1$H-NMR ($\Sigma$ H, M, J in MHz) (ppm): $\delta$ 3.51 (H-3); $\delta$ 5.36 d (5.38) (H-6); $\delta$ 0.69 s (H-18); $\delta$ 1.01 s (H-19); $\delta$ 0.92 d (6.5) (H-21); $\delta$ 0.88 d (6.85) (H-26); $\delta$ 0.90 d (6.85) (H-27); $\delta$ 5.82 dd (17.25; 10.76) (H-28); $\delta$ 5.20 dd (17.5; 1.5) (H-29); and $\delta$ 5.15 dd (10.75; 1) (H-29). Data obtained then compared to references concluded that isolate X1 is Saringosterol (Figure 1) [8].

![Figure 1. Structure of Saringosterol (Isolate X1)](image)

3.1.2. Isolate X2
Spectra of $^1$H-NMR ($\Sigma$ H, M, J in MHz) (ppm): $\delta$ 2.09 m (H-4); $\delta$ 2.06 m (H-5); $\delta$ 6.93 d (8.8) (H-14); $\delta$ 7.92 d (8.8) (H-15); $\delta$ 1.31 s (H-20); $\delta$ 2.84 s (H-21); $\delta$ 2.81 s (H-23); and $\delta$ 2.81 s (H-23). Data collected then compared to prediction reference by chemdraw 8.0 and reference, concluded that isolate X2 had similar structure to halenaquinone (Figure 2) [9].

![Figure 2. Structure of Halenaquinone (A) and Its Derivatives (B, C)](images)
3.2. Antibacterial Activity

Marine sponge *Xestospongia* sp. in this study found that isolate X1 is Saringosterol. Saringosterol is classified in the class of steroids. Previous studies showed that the class of steroid having acted in antibacterial. Steroid interacts with phospholipid membrane of the cell thus decreasing integrity and changing the morphological of cell membranes lead to the fragile and lysis of cell [10,11]. According to this study, isolate X1 (Saringosterol) was not active as antibacterial because it did not provide inhibition zone around the wells at each concentration (1000; 500; and 100 μg/mL used against both *S. aureus* and *E. coli* (Table 1).

Despite isolate X1 (Saringosterol) was unable to inhibit bacteria growth, isolate X2 (predicted compound-halenaquinone) has antibacterial activity observed by its inhibition zone around the wells (Table 1). At concentration 1000 μg/mL, X2 suggested intermediate susceptible against *S. aureus* (5.25 mm), and low susceptible against *E. coli* (4.5 mm), as well as at concentration 500 μg/mL, X2 suggested low susceptible against both *S. aureus* (3.75 mm) and *E. coli* (3.25 mm). Concentration of 100 μg/mL did not inhibit the growth of both bacteria. Isolate X2 suspected as halenaquinone belongs to quinone's class. Quinone acts as antibacterial by inhibits the bacteria's growth by forming irreversible complex isolate against nucleophilic amino acid in the transmembrane of the plasma membrane, the polypeptide of cell wall, and enzymes in cell wall, thus interrupting the bacteria’s life [11].

### Table 1. Inhibitory Zone (mm) of Isolate Saringosterol and Halenaquinone

| Isolate | Concentration (μg/mL) | *S. aureus* ATCC 25923 | *E. coli* ATCC 35218 |
|---------|-----------------------|------------------------|----------------------|
| X1      | 1000                  | -                      | -                    |
|         | 500                   | -                      | -                    |
|         | 100                   | -                      | -                    |
|         | Positive Controlᵃ     | 12.25 mm               | 15.5 mm              |
|         | Normal Controlᵇ       | -                      | -                    |
| X2      | 1000                  | 5.25 mm                | 4.5 mm               |
|         | 500                   | 3.75 mm                | 3.25 mm              |
|         | 100                   | -                      | -                    |
|         | Positive Controlᵃ     | 11.25 mm               | 13 mm                |
|         | Normal Controlᵇ       | -                      | -                    |

ᵃ: chloramphenicol;ᵇ: DMSO

4. Conclusion

Isolates which successfully isolated and identified from methanolic extract of marine sponge *Xestospongia* sp were saringosterol and halenaquinone. Saringosterol was not susceptible to both *Staphylococcus aureus* ATCC 25923 and *Escherichia coli* ATCC 35218 with various concentrations. Halenaquinone was susceptible at concentration of 1000 μg/mL with inhibition zone 5.25 mm against *S. aureus* and 4.5 mm against *E. coli*; as well as at concentration 500 μg/mL with inhibition zone 3.75 mm against *S. aureus* and 3.25 mm against *E. coli*. The study needs further identification by using spectra ¹³C NMR to determine the structures.
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References
[1] WHO. 2018. Antimicrobial resistance. Retrieved from: https://www.who.int/news-room/fact-sheets/detail/antimicrobial-resistance
[2] Sutradhar KB, Saha A, Huda NH, and Uddin R. 2014. Irrational Use of Antibiotics and Antibiotic Resistance in Southern Rural Bangladesh: Perspectives from Both the Physicians and Patients. *Annu Rev Bio.*, 4(9) 1421-30.
[3] Chasanah E. 2008. Marine Biodiscovery Research in Indonesia: Challenges and Rewards. *J. Coast. Dev.*, 12(1) 1-12
[4] El-Gamal AA, Shaza M, Lamia, Abdulrahman, Mansour, Abdelkader, Ashok, Maged S, Wael M, and Diaa. 2016. Cytotoxic Compounds from the Saudi Red Sea Sponge *Xestospongia testudinaria*. *Mar Drugs.*, 14(82) 1-9
[5] Wahyuni W, Fristiohady A, Malaka MH, Malik F, Yusuf MI, Leorita M, Sadarun B, Saleh A, Musmina WOS, Sabandar CW, and Sahidin I. 2019. Effects of Indonesian Marine Sponges Ethanol Extract on the Lipid Profile of Hyperlipidemic Rats. *J Appl Pharm Sci.*, 9(10) 001-8
[6] Matobole RM, van Zyi LJ, Parker-Nance S, Davies-Coleman MT, and Trindade M. 2017. Antibacterial Activities of Bacteria Isolated from the Marine Sponges *Isodictya compressa* and *Higginsia* bidentifera Collected from Algoa Bay, South Africa. *Mar Drugs.*, 15(2) 1-19
[7] Ankisetty S, and Slattery M. 2012. Antibacterial Secondary Metabolites from the Cave Sponge *Xestospongia sp.* *Mar Drugs.*, 10(5) 1037-43.
[8] Chen Z, Jiao L, Zhifei F, Cheng Y, Rhenshuai Z, Yiyun S, Ying Z, Haz Y, and Hongbing, L. 2014. 24 (S)-Saringosterol From Edible Marine Seawed *Sargassum fusiforme* Is a Novel Selective LXRβ Agonist. *J Agric Food Chem.*, 40
[9] Chao S, Caleb F, Marni B, John S, and David G. 2005. Halenaquinone and Xestoquinone Derivates Inhibitors Of Cdc25B Phosphatase From a *Xestospongia sp.* *Bioorg Med Chem.*, 13 999-1003
[10] Madduluri S, Rao KB, and Sitaram B. 2013. *In Vitro* Evaluation of Antibacterial Activity of Five Indigenous Plants Extract Against Five Bacterial Pathogens of Human. *Int J Pharm Sci.*, 5(4) 679-84
[11] Cowan MM. 1999. Plant Products as Antimicrobial Agents. *Clin Microbiol Rev.*, 12(4) 564-82