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Marine sponge *Jaspis sp*, a potential bioactive natural source against infectious diseases

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ABSTRACT

Puji Astuti, Sylvia Utami Tunjung Pratiwi, Triana Hertiani, Gemini Alam, Akbar Tahir, Subagus Wahyuono - ***Marine Sponge Jaspis sp, A Potential Bioactive Natural Source against Infectious diseases***

Background: The high incidence of microbial infection and the emergence of drug resistant and multidrug-resistant microbes as well as the lack of any current chemotherapy augmented the necessity to search for new and better antimicrobial drug. Marine invertebrates are known as rich sources of compounds with unique chemical structures and pronounced chemical and biological activities, which suggests potential value as lead structures for the development of new pharmaceuticals.
Objective: This study aims to screen potential antiinfective of sponges extracts collected from Barrang Lompo island and report on their antibacterial and antifungal properties.
Methods: Testing for anti-infective agents was conducted using dilution method. Nutrient Agar was used as the testing media and nutrient broth for the inoculation of microorganisms. *Staphylococcus aureus*, *Escherichia coli* and *Salmonella thypi* were used as the testing bacteria and *Candida albicans* for the testing fungi. Chloramphenicol was used as positive control for antibacterial testing and ketocozaole for antifungal testing.
Results: From the 11 acetone extracts tested, BL-02, BL-09, BL-10 and BL-12 was found to inhibit the growth of microorganisms and the extract of BL-10 was found to be the most active. Bioautography results suggest that the polar fractions were responsible for the growth inhibition.
Conclusion: the polar fraction of acetone extract of BL-10 was considered to be potential compounds for further characterization as anti infective agents.

Key words: screening, sponge, antibacteria, antifungi

ABSTRAK

Puji Astuti, Sylvia Utami Tunjung Pratiwi, Triana Hertiani, Gemini Alam, Akbar Tahir, Subagus Wahyuono - ***Spons Jaspis sp, Sumber Alam Bioaktif yang Potensial Melawan Penyakit Infeksi***

Latar Belakang: Tingginya kasus infeksi, meningkatnya kasus resistensi obat terhadap beberapa macam mikroba serta terbatasnya obat kemoterapi menunjukkan pentingnya penelitian terhadap senyawa yang baru dan lebih baik serta berpotensi sebagai antiinfeksi. Invertebrata laut diketahui merupakan sumber alam yang kaya akan senyawa baru dengan struktur yang unik dan mempunyai aktivitas kimia dan biologis; potensial untuk dikembangkan sebagai senyawa unggulan untuk pengembangan obat baru.
Tujuan: Penelitian ini bertujuan untuk melakukan skrining ekstrak spons yang berpotensial sebagai antiinfeksi yang dikoleksi dari pulau Barrang Lompo serta melaporkan aktivitas antibakteri dan antijamurnya.
Bahan dan cara: Uji antiinfeksi dilakukan dengan metode dilusi padat. Nutrient Agar digunakan sebagai media uji sedangkan nutrient broth sebagai media inokulasi mikroorganisme. *Staphylococcus aureus*, *Escherichia coli* dan *Salmonella typhi* digunakan sebagai bakteria uji dan *Candida albicans* sebagai jamur uji. Kloramfenikol digunakan sebagai kontrol positif antibakteri dan ketokonazol untuk antijamur.

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Hasil: Dari sebelas ekstrak yang diuji, empat ekstrak (BL-02, BL-09, BL-10 and BL-12) menghambat pertumbuhan microorganisme dengan BL-10 sebagai ekstrak yang mempunyai aktivitas tertinggi. Dari hasil bioautografi diduga bahwa fraksi yang bertanggungjawab terhadap penghambatan adalah fraksi yang mempunyai polaritas tinggi.
Simpulan: Fraksi polar dari ekstrak aseton BL-10 berpotensial sebagai kandidat eksplorasi lebih lanjut untuk penyakit infeksi.

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INTRODUCTION

The rise in the number of immunocompromised patients has led to an increase in bacteria and fungal infections. Beside the antibiotic resistance of bacteria, the non-susceptibility of fungal pathogens to classical drugs has become an increasing problems, particularly because currently available antimycotics are directed against the same limited number of targets within the fungi[1,2].

In early 1940s, when penicillin was first introduced for clinical therapy, the number of resistant strain is very few. However, this number continues to increase for several years from 21 strains in 1946 to 96-102 strains in 1978[3]. The intensity of endemic or epidemic infection in human and animals as well as the extent of the use of drug were suspected to be the major factors which determine the drug resistance.

Studies conducted in a Cicago hospital in 1952 found that some strains of *Staphylococcus aureus* were resistant to antibiotic erythromycin. The strains became resistant to a concentration of 100 mg/mL in which the sensitive strains were killed at the concentration of 1 μg/mL[3]. Similar studies were conducted by Kbarek[4] in Dr Sardjito Hospital in Yogyakarta. She found some strains of *Bacillus sp, E. coli, K. pneumoniae* and *Pseudomonas sp*, were resistant to some protein synthesis inhibitor-antibiotics and penicillin derivates.

Based on the increasing incidence of microbial infection and the continous emergence of microbial resistance to classical antibiotics, an urgent demand for a new class of anti-infectives has been formulated. Natural products are the major source of lead compounds for drugs against human pathogens. Marine invertebrates are known as rich sources of compounds having chemical and biological activities and promising to be developed as new pharmaceuticals. A diverse range of bioactivities of these natural resources have been reported which included insecticidal, antibacterial, antifungal and cytotoxic properties[5,6,7,8]. Analysis of the Philippine marine sponge Xestospongia ashmorica afforded four new manzamines compounds and reported to have antibacterial properties[9].

In this paper we screen potential antiinfective sponges extracts collected from Barrang Lompo island and report on their antibacterial and antifungal properties.

MATERIALS AND METHODS

Materials

The main source of sponges were collected from Barrang Lompo island, the extracts obtained were designated as BL-01 to BL-12; Nutrient Agar (Difco) was used as the testing media and nutrient broth for the inoculation of microorganisms. *Staphylococcus aureus*, Escherichia coli and Salmonella thypi were used as the testing bacteria and *Candida albicans* for the testing fungi. Chloramphenicol was used as positive control for antibacterial testing and ketoconazole for antifungal testing. The study was conducted in the Pharmaceutical Biology Laboratory, Faculty of Pharmacy, Gadjah Mada University in early 2002.

Methods

Extraction

The extracts were obtained by maceration using acetone pa (E.Merck). The solvent was evaporated using rotavapor (Heidolph WB 2000).

Antibacterial and antifungal testing

Testing for anti-infective agents was conducted using dilution method according to Mitscher *et al*[10]. Bacteria and fungi from slant agar were grown in nutrient broth (NB) and incubated for ± 24 hours. The colony was diluted in saline (NaCl 0,9%) until the turbidity similar to standard Mc. Farland (108 CFU) was obtained. The extracts were dissolved using DMSO (200 ml) and mixed with liquid form of nutrient agar (NA) (final volume of 10 ml). The mixture was poured into dishes and let to become solid; 5 ml of diluted colony was put on the solid medium in which the area have been marked and divided into four regions. The colony was spread using drigalsky (surface plate method). The dishes containing colony were incubated at 37°C for ± 24 hours. Chloramphenicol and ketoconazole were used as positive (+) controls with DMSO as the negative (-) one.

Bioautography

The most potent extract was examined using bioautography to determine the active fraction based on polarity. This was performed by Thin Layer Chromatography (TLC) followed by anti-bacterial and anti-fungal testing. The active fraction will show zone inhibition on the bacterial or fungal growth.

Species Identification

Identification of the species of the active marine sponge was conducted in the Faculty of Biology, Gadjah Mada University.

RESULTS AND DISCUSSION

The need for screening new drugs having potential antimicrobial activities continous to rise. As the number of incidence of microbial infection increases, the number of resistant or multiresistant microbes also rise. Isolation of antimicrobial compounds from terrestrial resources has been widely explored. From the past 30 years, the discovery of novel compounds from marine natural products have made some significant contributions toward the development of new pharmaceutical agents.

In this study screening for antibacterial and antifungal was performed using dilution method in

TABEL 1. - Anti bacterial and anti fungal activities of acetone extracts of sponges collected from Barrang Lompo island

Samples	Type of bacteria or fungi			
	S. aureus	*E. coli*	*S. typhi*	*C. albicans*
BL-01	--	--	--	+
BL-02	++	--	+	--
BL-03	--	--	--	+
BL-04	+	+	--	+
BL-05	--	--	--	--
BL-06	+	+	+	+
BL-07	+	--	--	--
BL-09	++	--	++	--
BL-10	++	++	++	++
BL-11	+	--	--	--
BL-12	++	+	--	+
Control (+) Chloramphenicol	++	++	++	++
Ketoconazole				++
Control (-) DMSO	--	--	--	--

Note: ++ = complete inhibition (no growth)
+ = low activity (partial growth, relative to the control)
-- = no activity (complete growth, relative to the control)

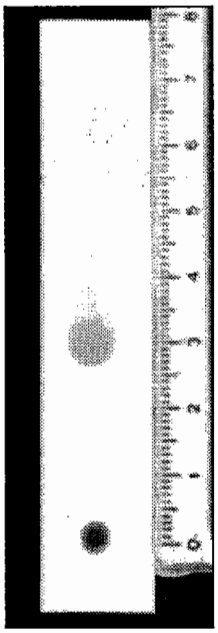

FIGURE 1. TLC profiles (chromatogram) of the acetone extract of BL-10 using detector cerium (IV) sulfate

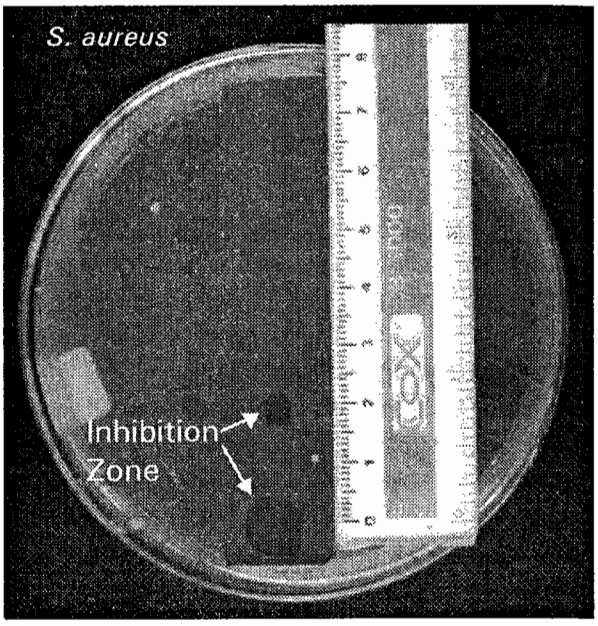

FIGURE 2. Inhibition zone of *S. aureus* growth from the acetone extract of BL-10 as identified by bioautography. Zone of inhibition was identified on the Rf of 0.28 and on the base spot of chromatogram

which the sponges extracts were mixed with nutrient agar media by the aid of small volume of DMSO. The extracts were obtained by maceration using acetone with the consideration that this solvent was able to extract non polar as well as polar compounds with minimally contaminated by sea salt.

According to Mitscher *et al*[10], an extract was considered to be active if it inhibits the growth of microorganism by the concentration of ≤ 1000 µg/ml. In this experiment, to avoid cross-contamination with the solvent (DMSO), negative control was made by growing the bacteria in the presence of DMSO (200 µl) without the extract. Chloramphenicol and ketoconazole were used as the positive controls and was given under concentration the same as the extracts, 1000 µg/mL.

By the given concentration (1000 mg/ml) from the 11 extracts tested, BL-10 was found to inhibit the growth of positive-gram bacteria (*S. aureus*), negative-gram bacteria (*E. coli*, *S. thypi*) as well as *Candida albicans* (TABLE 1). BL-09 inhibited the growth of *S. aureus* and *S. thypi* but not *E. coli* and *Candida albicans*. Although BL-02 and BL-12 extracts showed growth inhibition of positive-gram bacteria, these extracts did not inhibit the growth of negative-gram bacteria. Structure of the cell wall of negative-gram bacteria was suggested to influence the permeation of large molecule into the cell. Besides consisting of peptidoglycan and teicoat acid, the structure of negative-gram bacteria contains other three polymers, i.e. lipoprotein, exomembrane and liposacharide, whilst positive-gram bacteria consists of peptidoglycan and teicoat acid only[11].

The ability of sponges BL-10 extract to inhibit the growth of bacterial tested showed a significant value for the effort of finding novel antimicrobial compounds. As we know that *S. aureus*, *E. coli* and *S. thypi* are major types of organisms cause diseases in humans such as diarrhea, skin infection and thyphoid fever. Those bacteria are widely found in food or water contaminated by feces of infected humans or animals. On the other hand, BL-10 extract was also able to inhibit the growth of pathogenic fungi *Candida albicans*, the microbial cause mycosis candidiasis. This fungi is a member of the normal microbiota within the vaginal area, gastrointestinal tract, respiratory tract and mouth.

Further exploration of this finding will give a great contribution to the discovery of antifungal drug leads.

Since compared to other BLs the acetone extract of BL-10 was considered to be the most active, this extract was further examined for characterization. To determine the active fractions/ compounds, bioautography was performed. This method is able to detect active fractions/compounds based on the polarity. The fractions/compounds of the most active extracts were separated by Thin Layer Chromatography (TLC) with appropriate mobile and stationary phase. N-hexane: ethyl acetate = 3:1 was found to be the best mobile phase with silica gel GF254 as the stationary phase (FIGURE 1). The separated fractions or compounds were tested for their antibacterial or antifungal properties.

The polar fraction of BL-10 acetone extract was found to be responsible for the inhibition of the growth of microorganisms. Inhibition zone was observed at the Rf of 0.28 and at the base spot of chromatogram when the activity was tested using *S. aureus* (FIGURE 2). Although in the preliminary screening this extract inhibited all microorganisms tested, in this experiment no inhibition zone was observed when other microorganisms were used. Besides the complexity of the cell wall of negative-gram bacteria which was considered to play the role, these results suggest influence of the concentration of the compounds in the extract. As an extract contained a number of compounds which have different quantities, the highest quantity of compound was suggested to dominate the activities of others. Another possible reason is that the concentration of the sample tested for TLC was too small that unable to inhibit the growth of microorganism tested. Based on its morphology, the tissue and the specula, the active extract (BL-10) was identified as *Jaspis sp.*

CONCLUSION

1. Acetone extract of BL-10 compared to other BLs was found to be the most active extract against microorganism tested in which the species was further identified as *Jaspis sp.*
2. The polar fractions were suggested to be the ones which are responsible for the inhibition of the growth of *S. aureus*.
3. These fractions were considered to be potential for further characterization as antiinfectious drug leads.

SUGGESTION

Fractionation and isolation are needed to determine the active antiinfectious compounds.

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