Marine Sponge *Jaspis sp*, A Potential Bioactive Natural Source against Infectious diseases

Fuji Astuti, Sylvia Tunjung Utami, Triana Hertiani, Gemini Alam, Akbar Tahir, Subagus Wahyuono

1 Faculty of Pharmacy, Gadjah Mada University, Yogyakarta
2 Department of Pharmacy, Faculty of Natural Science, University of Hassanudin, Makasar
3 Coral Research Center, University of Hassanudin, Makasar

ABSTRACT

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

Background: The emergence of drug resistant and multidrug-resistant TB and malaria strains as well as the lack of any current chemotherapy augmented the necessity to search for new and better anti-TB, malaria, toxoplasmosis and other anti-infective drug leads

Objective: This study aims to screen potential anti infective 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 thipii* 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 anti fungal testing.

Results: From the 11 acetone extracts tested. BL-02, BL-09, BL-10 and BL-12 was found to inhibit the erowth of microorganisms and the extract of BL-10 was found to be the most active. Autobiography 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, anti bacteria, anti fungi

ABSTRAK

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

Latar Belakang: Meningkatnya kasus resistensi obat dan resistensi kembiasan obat untuk TB dan malaria serta terbatasnya obat kemoterapi yang baru menunjukkan pentingnya penelitian terhadap senyawa yang baru dan lebih baik serta berpotensi sebagai anti-TB, malaria, toxoplasmosis dan penyakit infeksi lainnya.

Tujuan: Penelitian ini bertujuan untuk skrining ekstraks yang berpotensial sebagai anti infeksi yang dikoleksi dari pulau Barrang Lompo serta melaporkan aktivitas anti bakteri dan anti jamurnya.


Hasil: Dari sebelas ekstrak yang diuji, empat ekstrak (BL-02, BL-09, BL-10 and BL-12) menghambat pertumbuhan bahwa fraksi yang heranppiina iawah terhadan nenphambatan adalah fraksi vaatJ memnnvai nolaritas tinfwi

Simpulan: Fraksi polar dari ekstrak aseton BL-10 berpotensial sebagai kandidat eksporasi lebih lanjut untuk penyakit infeksi.

Kata Kunci: skrining, spon, anti bakteri, anti jamur
INTRODUCTION

The rise in the number of immunocompromised patients has led to an increase in bacteria and fungal infections. As microbial resistance to classical antibiotics has emerged continuously, an urgent demand for a new class of anti infectives has been formulated. 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.

In 1998, the most dangerous malaria parasite, Plasmodium falciparum which causes cerebral malaria, is expected to spread in the central or northern regions of Europe and North America within a few decades. The pathogenic synergy with HIV has increased the overall incidence of many opportunistic infectious diseases. For example, the incidence of tuberculosis infection in HIV positive cases is 50-fold over HIV negative ones and the same holds true in the case of T. gondii.

The emergence of drug resistant and multidrug-resistant TB and malaria strains as well as the lack of any current chemotherapy augmented the necessity to search for new and better anti-TB, malaria and toxoplasmosis drug leads.

Analysis of the Philippine marine sponge Xestospongia ashmorica afforded four new manzamines compounds and reported to have antibacterial properties. Manzamin A which previously received considerable interest because of its potential as anti cancer agents, inhibiting malaria parasites not only in vitro but also in vivo.

In this paper, we screen potential anti infective 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 extract obtained were designated as BL-01 - 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 thyphii 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 anti-fungal testing.

Methods

The extracts are obtained by maceration using acetone pa (E.Merck). The solvent was evaporated.

Testing for anti-mfective agents was conducted using dilution method according to Mitscher et al. (1972). Bacteria and fungi from slant agar were grown in nutrient broth (NB) and incubated for 24 hours. Mc. Farian (10 CFU) was obtained. The extracts were dissolved using DMSO (200 ul) 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 ul 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 dragalsky (surface plate method). The dishes containing colony were incubated at 37°C for + 24 hours. Chloramfenicol and ketoconazole were used as positive (H) 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 antibacterial 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

Screening for anti bacterial and anti fungal was performed using dilution method in which the
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., 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.

**Table 1. Anti bacterial and anti fungal activities of acetone extracts of sponges collected**

<table>
<thead>
<tr>
<th>Samples</th>
<th>S. aureus</th>
<th>E.coli</th>
<th>S. typhi</th>
<th>C. albicans</th>
</tr>
</thead>
<tbody>
<tr>
<td>BL-01</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>BL-02</td>
<td>++</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>BL-03</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>BL-04</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>BL-05</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>BL-06</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>BL-07</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>BL-09</td>
<td>-</td>
<td>-</td>
<td>++</td>
<td>-</td>
</tr>
<tr>
<td>BL-10</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>BL-11</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>BL-12</td>
<td>++</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Control (+)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chloramphenicol</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>Ketoconazole</td>
<td></td>
<td></td>
<td></td>
<td>++</td>
</tr>
<tr>
<td>Control (-)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DMSO</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Note:  
++ = complete inhibition (no growth)  
+ = low activity (partial growth, relative to the control)  
~ = no activity (complete growth, relative to the control)
By the given concentration (1000 pg/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. thipii) as well as Candida albicans (table 1). BL-09 inhibited the growth of S. aureus and S. thipii 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. Since the acetone extract of BL-10 was considered as the most active one, 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 anti bacterial or fungal properties.

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

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

ACKNOWLEDGEMENT

We thank to Kirlan, S.Si and Jusain Setiadi, S.Si (University of Hassanudin, Makasar) for assistance with sample collection. This work was partly supported by DIKS Faculty of Pharmacy, Gadjah Mada University, SK. No. 3760/P/FA/2002, Date 1 July 2002.

REFERENCES