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Short Communication

Antimicrobial activity of endophytic fungi from marine Sponge *Haliclona fascigera*

Dian Handayani, Rizka Fauza Ahdinur, R. Rustini

Faculty of Pharmacy, Andalas University, Padang 25163, Indonesia.

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ABSTRACT

Fungi associated with marine sponge *Haliclona fascigera* has shown a promising source in the search for new antimicrobial compounds. We have isolated 25 endophytic fungi from the sponge, which were cultured in Sabouraud Dextrose Broth (SDB) for 4 weeks at 25-27ºC. The ethyl acetate extracts of the isolates-broth were then tested for its antimicrobial activity against *Staphylococcus aureus*, *Escherichia coli*, and *Candida albicans* by using agar diffusion method. The zone of inhibition was measured and expressed in millimeters. There were 8 isolates of the fungi that considered active to *Staphylococcus aureus*, i.e. HF2 isolate (14,5 mm), HF8 isolate (12,5 mm), HF9 isolate (14 mm), HF13 isolate (16,5 mm), HF14 isolate (11 mm), HF19 isolate (14,5 mm), HF21 isolate (14,5 mm), HF22 isolate (13 mm), and 1 isolate active to *C. albicans*, i.e. HF16 isolate (12,5 mm). The identification of the bioactive fungi isolates was done by comparing its macroscopic and microscopic characteristic based on literature. From the study, it can be concluded that fungi associated with marine sponge *Haliclona fascigera* possesses potentially antimicrobial activity which might be due to the presence of bioactive metabolite compounds.

INTRODUCTION

Marine sponges are a filter-feeding organism, which form close associations with a wide variety of microorganism. Approximately40% of sponge biomass is estimated to contain bacterial communities. These bacteria are symbionts in the body sponge. Several studies have shown that the symbionts has a role in the production of bioactive compounds which serves in the ecological adaptation of sponge (Proksch *et al*., 2003; Thakur & Muller, 2004; Zheng *et al*., 2005). However, sponges are not only rich in bacteria, but also contain dense and highly diverse of symbiotics fungi. Marine bacteria and fungi have shown to be potential as new promising sources of a huge number of bioactive secondary metabolites. Some of these marine species life in a stressful habitat, under cold, lightless and high pressure condition (Debbab *et al*., 2010). Bioactive secondary metabolites from sponges associated with microorganisms have shown activity as anticancer, antibacterial, antifungal, antiviral, antiprotozoal, anthelmintics, anti-inflammatory, neurosuppresive, imunosuppressive, and antifouling (Vasanthabharathi and Jayalakshmi, 2011; Alexander, *et al*. 2012). The marine sponge *Haliclona fascigera* is chosen as host of isolated fungi. Previous investigation has shown that this sponge was a potential source of antimicrobial metabolites compounds. The present research study aims in isolation various fungi from marine sponge *Haliclona fascigera* and to evaluate their antimicrobial activity by performing antibacterial studies.

MATERIAL AND METHODS

Sponge material

H. fascigera was collected from the Mandeh island, South Coast of West Sumatera, Indonesia, in the depth of ± 5m. The sponge put soon after collection to a sterile plastic bag and stored in the ice box for the isolation of endophytic fungi and transported to the laboratory.

Isolation of Fungi associated with marine sponge

Isolation of endophytic fungi begins with a sample surface sterilization. Sponge was rinsed with sterile seawater, then cut into small pieces, taken about 10 grams and put in to erlenmeyer flask and add 100 ml sterile seawater.

* *Corresponding Author*
Dian Handayani, Faculty of Pharmacy, Andalas University, Padang 25163, Indonesia. Email: mail id: dianh_17@yahoo.com

Then it was diluted until its concentration 10^{-4} and inoculated by pour plate method, taken about 1 ml from each dilution to inoculate on (Sabouraud Dextrose Agar) SDA medium in the petridish ± 15ml aseptically then incubated at temperature 37 °C for 5-7 days. Colonies that have a different shape to the other colonies can be regarded as different isolates. Then be purified to obtain pure isolates (single fungi).

Cultivation of fungi isolates for antimicrobial activity screening

Pure isolates obtained in the insulating phase and then cultured in media SDB, pure isolates taken one loop, then put in a 10 ml SDB medium and incubated at room temperature for 7 days. Furthermore, isolates were grown on a scale of 10 ml were transferred aseptically similar to the culture medium at a larger scale (100 ml) and incubated at room temperature for 3-4 weeks.

Extraction of secondary metabolites from fungi isolates

Pure isolates that had been grown for 3-4 weeks, then extracted by maceration with ethyl acetate (EtOAC) in the ratio of 2:1. After macerated overnight, the fungal mycelium was then split using a sonicator for 5 minutes. The cultures were then filtrated by whatman paper. Furthermore, the ethyl acetate extract was separated from the culture medium using a separating funnel. This organic solvent was pooled and then taken to dryness using a vacuum rotary evaporator at 40ºC.

Screening for antimicrobial activity

For screening of antimicrobial activity, the EtOAc extract of endophytic fungi was tested against *Staphylococcus aureus*, *Escherichia coli*, and *Candida albicans* using the paper disk method. One pieces of 6 mm sterile paper disk was soaked in each of EtOAc extract (10 mg/ml in DMSO).Paper disks were also inoculated with DMSO (negative control), Oxytetrasiklin (20 $mg.ml^{-1}$ in distilled water) and Clotrimazol (500 $\mu g.ml^{-1}$ in distilled water) as positive controls. The disk was placed on the surface of the medium containing 10^5cell of bacteria and fungi test strain. The plates were incubated at 37 °C for 24 hours for bacteria and at a temperature of 25 °C-27 °C for 5-7days for fungi. The width of inhibition zones was measured. Each treatment consisted of three replicates. The experiment was repeated twice.

RESULTS AND DISCUSSION

In this study, we have isolated 25 endophytic fungi from marine sponge *H. fascigera*. The EtOAc extracts of endophytic fungi were tested for its bioactivity against microbial pathogens such as *S. aureus*, *E. coli* and *C. albicans* by agar diffusion method. Based on the test results of the antimicrobial activity of 25 extracts fungal isolates in Table 1, eight isolates can inhibit the growth of *S. aureus* with halos between 11 and 16.5 mm. All extracts were inactive against the Gram-negative bacteria *E. coli*. Usually, Gram negative bacteria are less sensitive to antibiotic substance due to their cell membrane structure is different from Gram positive bacteria. Only one extract can inhibits the growth of pathogenic fungi *C. albicans*. The most effective extract against *S. aureus* was HF13.

Table 1: Antimicrobial activity of endophytic fungi extracts of *H. fascigera* against human pathogenic .

Fungi Extract	Zone of inhibition (mm)		
	Escherichia coli	***Staphylococcus aureus***	***Candida albicans***
HF1	-	-	-
HF2	-	**14.5**	-
HF3	-	-	-
HF4	-	-	-
HF5	-	-	-
HF6	-	-	-
HF7	-	-	-
HF8	-	**12.5**	-
HF9	-	**14.0**	-
HF10	-	-	-
HF11	-	-	-
HF12	-	-	-
HF13	-	**16.5**	-
HF14	-	**11**	-
HF15	-	-	-
HF16	-	-	**12.5**
HF17	-	-	-
HF18	-	-	-
HF19	-	**14.5**	-
HF20	-	-	-
HF21	-	**14.5**	-
HF22		**13**	
HF23	-	-	-
HF24	-	-	-
HF25	-	-	-

Fungal endophytes were selected for futher characterization and identification based on Brigitte (1980). Macroscopic examination includes a visual observation to the form colony or hyphal, surface and reverse colony color, and colony texture. While on microscopic examination was carried out by observing the characteristic of the spores or conidia, and reproductive structures (sexual and asexual) under a light-field microscope. Based on the results of morphologic identification of a total of nine isolates of fungi that show antimicrobial activity, seven of which are the same type of fungi, the isolate of HF2, HF8, HF9, HF13, HF19, HF21 and HF22.From the results of microscopic examination of three isolates of fungi that have antimicrobial activity showed that the isolated fungus HF13 was *Aspergillus niger*, HF14 was a fungus of *Candida* sp, and HF16 was unidentified. Fenical and Jensen (2000) reported that micro fungi *Aspergillus niger* was a fungal symbionts on marine sponge *Hyrtios* sp. And in this research, it was obtained seven similar fungi of *Aspergillus niger* so it can be concluded that *Aspergillus* was a major microfungal on *H. fascigera*. According to Brigitte (1980), the following antibiotics produced also by one species or genus *Aspergillus* and *Penicillium*. Antibiotics produced by *Aspergillus* include Candidulin, Fumagallin, Fumigatin, Geodin, Helvolinsaure, Mellein, Nidulin, Nornidulin, Terein and Tereinsaure. Recently, it was reported the isolation of cytotoxic compounds, asperazine, from *Aspergillus niger* strains obtained from sponge *Hyrtios* sp. Asperazine is diketopiperazinea

symmetric dimer containing tryptophan modification unit. These compounds, related to the same aminoacidare reported from terrestrial Fungi, showed significant activity in leukemia with the cytotoxicity of 50mg/disc. Asperazine shown inactive against *B. subtilis* or *C. albicans* (Jensen and Fenical, 2000). From the results of the bioactivities conducted to theFungiisolatesHF13, it shown also inactive against *C. albicans* but only active against *S. aureus*. The difference of bioactivity produced by the fungus *A. niger* may be caused by differences in waters where taking a sponge and different kind of host. According to Bell and Barnes (2003), morphology and physiology sponge influenced by the microenvironment of a life. Growth and metabolism as well as symbionts associated with the sponge will also be influenced by these factors. Bell and Barnes (2003) also stated that the growing substrate can affect the morphology sponge, thus will also affect its bioactivity. Factors that may affect the morphological adaptations sponge include water flow, sedimentation and substrate type. Their research also showed that these factors may lead to differences in morphology and bioactivity significantly in one habitat.

CONCLUSIONS

From the results of this research, the antimicrobial activity shown by endophytic fungi from *H. fascigera* might be due to the presence of its secondary metabolites. It can also be conclude from the present study that endophytic fungi might be used as an alternative to produce the antibiotic used in pharmaceutical on the basis inhibition of pathogenic microorganisms. However, further research needs to be done in determining antibiotic compounds produced by those endophytic fungi.

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