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ANTIBACTERIAL INVESTIGATED OF PHOSPHOLIPASE A_2FROM THE SPINES VENOM OF CROWN OF THORNS STARFISH *ACANTHASTER PLANCI*

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ABSTRACT

Phospholipase A2 isolated from venoms is a potential new antibacterial source. Antibacterial activity tests of phospholipase A_2 were performed against *Staphylococcus aureus* and *Bacillus subtilis*. Phospholipase A_2 which was isolated from spines venom of *Acanthaster planci* by using fractionated 20% saturated ammonium sulfate precipitation and its antibacterial assay was done by using disc diffusion method whereas 40 µl isolate at a concentration of 6.29 µg/µl was applied onto 7 mm paper disc. The assay exhibited inhibition zone around the phospholipase A_2disc against *Staphylococcus aureus* while no inhibition zone against *Bacillus subtilis*. As a result, phospholipase A_2 posses an antibacterial activity against *Staphylococcus aureus*.

KEYWORD: **Phospholipase A_2, venom, *Acanthaster planci*, antibacterial activity**

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INTRODUCTION

Antibiotic resistance has been a great concern during the last decades due to the extensive clinical use of classical antibiotics (Ahmadi *et al*., 2010). The bacteria such as high resistance of *Staphylococcus aureus* against - chloramphenicol, ciprofloxacin and amoxicillin, methicillin resistant *Staphylococcus aureus* (MRSA), *Pseudomonas, Klebsiella, Entrobacter, Acinobacter, Salmonella, Staphylococcus, Enterococcus* and penicillin-resistant *Streptococcus pneumoniae* (PRSP) and vancomycin-resistant *Enterococci* - have developed several ways to resist antibiotics. Searching result for antimicrobial agents from several different sources found a result of discovery of new effective antibacterial agents or developing antibacterial with a new mechanism is continuously necessary (Bonomo, 2000; Nafeesa *et* al., 2001; Ang et al., 2004). Natural products are an important source of medicinal compounds. Variety organisms produce such bioactive compounds and some of the substances have been shown to be able to kill bacteria (Wenhua *et al*., 2006; Jensen *et al*., 2006; Samy *et al*., 2006;2007; Shittu *et al*., 2007;Chellapandi and Jebakumar, 2008). Phospholipase A_2 purified from snake venom possess antibacterial activity against *Staphylococcus aureus, Proteus vulgaris, Proteus mirabilis,* and *Burkholderia pseudomallei* (Samy *et al*., 2006; 2008). Phospholipase A_2would be prospective for antibacterial agents, especially against drug resistant bacteria. *Acanthaster planci* is a potential source of phospholipase A_2. Previously, phospholipase A_2 successfully purified from spines venom of *Acanthaster planci* by using rapid and simple method. Purification was performed by fractionated preheated crude venom by using 20% saturated ammonium sulfate precipitation and produced single band protein with specific activity of phospholipase A_2 twenty times crude venom (Savitri *et al*., 2011). This present research proposed to confirm the antibacterial activity of phospholipase A_2 from *Acanthaster planci* spines venom against *Staphylococcus aureus* and *Bacillus subtilis*.

MATERIALS AND METHODS

Acanthaster planci

The specimen was captured from the East Ambon bay at Mollucas islands, Indonesia in April 2012, and live specimen from the field immediately frozen and stored at -20 ^{o}C before using.

Isolation of Phospholipase A_2

Isolation of phospholipase was done as Savitri *et al* (2011) method. Firstly, extracted crude venom was found from 50 grams of spines which was sonicated in 100 ml 0,01 M phosphate buffer (pH 7.0) and 0.001 M $CaCl_2$ and then was centrifuged for 30 minutes at 15,000 G at 4 ^{o}C, and furthermore, its supernatant called crude venom was harvested. Secondly, heated venom was found from harvested crude venom which was heated for 30 minutes at 60 ^{o}C and then centrifuged at 15,000 g and 4 ^{o}C for 30 minutes and furthermore, its supernatant called heated venom was harvested. Thirdly, isolated of phospholipase $A_2$from heated venom was found by using 20% saturated ammonium sulfate precipitated and then was centrifuged at 30,000 g and 4 ^{o}C. Furthermore, precipitated phospholipase A_2 (PLA2) was dissolved in 0.01 M phosphate buffer pH 7.0 and 0.001 M $CaCl_2$ sufficiently and stored at 4^{o}Cuntil use for any tests.

Phospholipase A_2 Activity Assay

PLA2 activity assay was done according to the Marinetti method (1965), which its analysis was perform by measuring the clearing of egg-yolk suspension. Egg yolk suspension is made in 0.01 M phosphate buffer (pH 7.0) at a concentration of 2 mg egg yolk/ml. Absorbance which was measured at 900 nm, is performed to mixture of 0.2 ml of stored PLA2 and 3 ml egg yolk suspension in 5 minutes interval. Enzyme activity causing the decrease of 0.01 in absorbance/minute was defined as1 (one) unit.

Determination of Protein Concentration

Protein concentration was determinate by Lowry method (1951), using bovine serum albumin (BSA) as a standard.

Antibacterial Activity Assay

Bacterial strain *Staphylococcus aureus* (FDA 209P) and *Bacillus subtilis* (PCI 219) and broad-spectrum standard antibiotic chloramphenicol was used in this research given by Research center for Biology, Indonesian Institute of science, Cibinong Bogor, Indonesia. Antibacterial activity assayed by using Disc Diffusion Method according to Bauer *et al* (1966) with modification. Bacterial inoculums were spread onto sterile NA agar plates (90-mm diameter), then it allowed till surface medium were solid and dry. Furthermore, sterile paper discs (7-mm diameter) were placed onto the nutrient agar surface and addition of 40 µl of phospholipase A_2 at a concentration of 2.98 µg/µ1, chloramphenicol (30 µg/disc) as positive control and 0.01 M phosphate buffer (pH 7.0) pre-added with 0.01 M $CaCl_2$as normal control, was performed. Hereinafter, prior to used, PLA2, chloramphenicol and phosphate buffer-$CaCl_2$were filtered (0.22 µm). These plates were incubated at 37°C for 24 hours, followed by measured and calculated of diameter of inhibition zones minus the disc diameter. This assay activity was performed to obtain 4 (four) replicated results.

RESULTS AND DISCUSSION

Isolation of PLA2 was performed as Savitri *et al* (2011). Fractionated preheated venom by using 20% saturated ammonium sulfate precipitation, produced PLA2 with the specific activity of 15.39 units/mg and a protein concentration of 2.98 µg/µl. The PLA2 specific activity of crude venom was 1.66 unit/mg with a protein concentration of 6.29 µg/µl. The specific activity of PLA2 was increased fifteen times comparing with crude venom, and the yield obtained was 0.9%. This PLA2 yield obtained was slightly less than Savitri *et al*(2011) which was reported previously, and also with a slightly lower specific activity of PLA2. Inconsistently of yield quantity and specific activity of PLA2 was influenced by its ecology and physiology factors such as season, feeding and age whereas implicated in individual gene expressing. In order to evaluate antibacterial effect of PLA2 from *Acanthaster planci* spines venom, disc diffusion assay was carried out with 40 µl (6.29 µg/µl) of phospholipase A_2, antibiotic standard chloramphenicol (30 µl/disc) as positive control and 0.01 M phosphate buffer pH 7.0 pre-added 0.01 M $CaCl_2$ were performed, and result was shown at Figure 1.

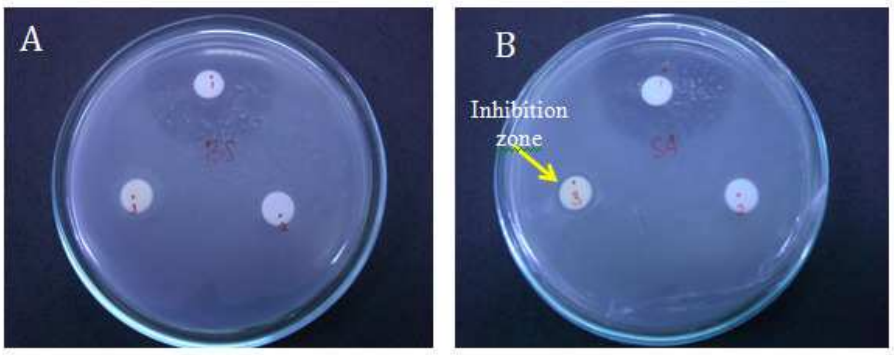

Figure 1
Antibacterial activity assay, A. Bacillus subtilis, B. Staphylococcus aureus (1) Chloramphenicol; (2) Phosphate buffer-$CaCl_2$; (3) PLA2 - Phospholipase A_2

Result assay shown a narrow inhibition zone (1.5 mm) around of PLA2 disc against *Staphylococcus aureus* (B, 3) whereas relatively small comparing with chloramphenicol (25 mm, B, 1). Although its inhibition zone relatively small, it indicated that

PLA2 from spines venom of *Acanthaster planci* posses a potent antibacterial activity against *Staphylococcus aureus*. According to Samy *et al* which was previously reported, PLA2 from spines venom of *Acanthaster planci* posses antibacterial activity against *Staphylococcus aureus* is similar to snake venom based PLA2. Here, snake venom based PLA2 exert its bactericidal effect by permeabilizing the bacterial membrane by forming pores. This snake venom based PLA2 displays a bactericidal effect, which may be either dependent or independent of catalysis. Otherwise, PLA2 did not exhibited inhibition zone against *Bacillus subtilis*. Similar to *Echis carinatus* venom extracted PLA2 activity result which also shown no antibacterial effect against *Bacillus subtilis* and as a conclusion, its venom PLA2 does not guaranteed have antibacterial activity against *B. subtilis* (Ahmadi *et al.,* 2010). Based on the above result it concluded that *Acanthaster planci* spines venom based PLA2 was posses antibacterial activity against *Staphylococcus aureus* and it is potential to develop this PLA2 into an antibacterial drug agent.

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