

# Pemanfaatan phospholipase A2 racun duri bintang laut acanthaster planci untuk bahan antimikroba = Utilization of phospholipase A2 of the crown of thorns acanthaster planci spines venom for antimicrobial agents / Imelda Krisanta Enda Savitri

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## Abstrak

[<b>ABSTRAK</b><br>

Racun duri Acanthaster planci memiliki beragam aktifitas biologi yaitu aktifitas lethal, aktifitas hemolitik, aktifitas myonecrotic, aktifitas pendarahan, peningkatan aktifitas permeabilitas kapiler, aktifitas edema, aktifitas phospholipase-A2 (PLA2), aktifitas pelepasan histamin dari mast cell dan aktifitas kardio vaskular. Racun duri Acanthaster planci mengandung phospholipase A2 (PLA2), plancitoxin yang homolog dengan deoxyribonuklease II pada mamalia dan plancinin peptida antikoagulan.

Berbagai penelitian terdahulu membuktikan bahwa racun yang berasal dari berbagai hewan mengandung senyawa yang potensial dikembangkan sebagai bahan antibiotik dan terapeutik untuk mengobati suatu penyakit. Dengan potensi aktivitas biologi tersebut racun Acanthaster planci dapat berkontribusi di bidang medis yang bisa menjadi masukan bagi pendapatan negara. Efek antimikroba hasil aktifitas hidrolisis komponen fosfolipid membran sel mikroba oleh enzim PLA2 dapat bermanfaat bagi pengembangan bahan antibiotik. PLA2 yang dimurnikan dari racun ular memiliki aktifitas antibakteri terhadap Staphylococcus aureus, Proteus vulgaris, Proteus mirabilis, and Burkholderia pseudomallei. Selain itu, PLA2 memiliki aktifitas antiHIV melalui mekanisme penghambatan pelepasan intraseluler protein capsid virus dan diasumsikan PLA2 memblok virus masuk ke dalam sel inang sebelum virus tersebut membuka selaputnya dan secara independent memanfaatkan koreseptornya. PLA2 melindungi sel limfosit T manusia dengan memblok virus yang memiliki selubung luar mengandung fosfolipid.

Acanthaster planci merupakan predator yang mengancam populasi karang terutama ketika terjadi peledakan populasi. Pemanfaatan Acanthaster planci untuk produksi PLA2 dapat menjadi alternatif produktif upaya pengendalian populasinya sekaligus membuatnya menjadi lebih berguna. Purifikasi PLA2 racun duri Acanthaster planci telah dilakukan oleh Shiomi dan koleganya menggunakan rangkaian kolom kromatografi bertingkat, memerlukan biaya yang relative mahal dan membutuhkan waktu beberapa hari, sehingga dalam penelitian ini dikembangkan metode purifikasi yang sederhana dan cepat dengan biaya yang relatif murah. Hasil penelitian ini diharapkan dapat menjadi masukan bagi upaya pemanfaatan Acanthaster planci untuk menghasilkan PLA2 yang berpeluang dikembangkan sebagai bahan antibakteri dan antiHIV.

Penerapan metode percobaan yang dilakukan dalam penelitian ini memberikan hasil sebagai berikut :

&#61623; Proses ekstraksi racun dari jaringan duri Acanthaster planci berlangsung efektif

melalui proses sonikasi pada 20 kHz selama 2x8 menit (intensitas 80% dan output 10). Racun yang terekstraksi tertampung dalam larutan 0,01 M bufer fosfat pH 7,0 mengandung 0,001 M CaCl<sub>2</sub> yang digunakan sebagai media ekstraksi disebut crude venom. Pengujian secara kualitatif menggunakan darah manusia yang diberi perlakuan crude venom (1:1) memperlihatkan antikoagulasi darah oleh plancinin yang terkandung dalam racun membuktikan keberhasilan proses ekstraksi. Pada awalnya dilakukan pula metode ekstraksi dengan cara duri diblender terlebih dahulu dan dilanjutkan dengan disonikasi. Untuk meminimalisir protein kontaminan yang berasal dari jaringan duri dan mempertimbangkan efisiensi maka metode ini kemudian tidak diterapkan.

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Purifikasi phospholipase A<sub>2</sub> racun duri *Acanthaster planci* dari Ambon-Maluku melalui pengendapan amonium sulfat bertahap pada tingkat kejenuhan 20% terhadap crude venom yang telah dipanaskan efektif memurnikan PLA<sub>2</sub>. Hasil elektroforesis SDS-PAGE memperlihatkan isolat PLA<sub>2</sub> memiliki satu pita protein sedangkan crude venom memiliki empat pita protein. Isolat PLA<sub>2</sub> yang dihasilkan memiliki aktifitas spesifik 20 kali aktifitas spesifik crude venom. Pemanasan crude venom pada 60°C selama 30 menit yang diikuti dengan sentrifugasi selama 30 menit pada 15.000xg dan 4°C memisahkan protein tidak tahan panas dari PLA<sub>2</sub>. Metode purifikasi ini juga diterapkan pada racun duri *Acanthaster planci* dari Sorong-Papua namun belum berhasil. Sedangkan purifikasi PLA<sub>2</sub> melalui pengendapan menggunakan etanol dengan tingkat kejenuhan 80% tidak efektif memurnikan PLA<sub>2</sub> namun dapat meningkatkan aktifitasnya menjadi lima kali aktifitas crude venom. Hasil eksperimen ini dipublikasikan di *International Journal of Pharma and Bio Science* Vol 2/issue 2/Apr-Jun 2011 and *International Journal of Pharma and Bio Science* 2012 Oct; 3(4):(B) 603-608

&#1623; Pengujian aktifitas antibakteri menggunakan metode difusi cakram memperlihatkan terbentuknya zona bening disekitar cakram PLA<sub>2</sub> pada kultur *Staphylococcus aureus* yang mengindikasikan bahwa PLA<sub>2</sub> racun duri *Acanthaster planci* memiliki aktifitas antibakteri terhadap *Staphylococcus aureus* pada dosis 2,98 mg/ml. Hasil eksperimen ini dipublikasikan pada *International journal of Pharma and Bio Science* 2013 Apr; 4(2) : (B)1-5

&#1623; Pengujian aktifitas antiHIV secara kualitatif menggunakan PBMC pasien HIV (ODHA) memperlihatkan terjadinya penurunan intensitas pita protein DNA pada hasil elektroforesis RT-PCR RNA sampel kultur HIV yang diberi perlakuan PLA<sub>2</sub>.

Selanjutnya analisis kuantitatif hasil Green Fluorescence Particle memperlihatkan terjadinya penurunan jumlah sel yang terinfeksi HIV secara signifikan oleh perlakuan PLA<sub>2</sub> dari 9,72% menjadi 0,29% yang mengindikasikan PLA<sub>2</sub> racun duri *Acanthaster planci* memiliki aktifitas antiHIV. Hasil eksperimen ini dipublikasikan pada *Asian Pacific Journal of Tropical Medicine* (2014) 412-420

&#1623; Biaya purifikasi PLA<sub>2</sub> merupakan pembiayaan yang dibayarkan untuk 1) bahan kimia dan peralatan habis pakai, 2) listrik untuk operasional alat, 3) sewa peralatan dan 4)

tenaga kerja. Hasil perhitungan biaya isolasi-purifikasi PLA2 menghasilkan nilai Rp. 446.192,- per 50 gram duri dengan hasil yang diperoleh adalah 4,622 mg PLA2. Biaya purifikasi PLA2 miniscale yang dilakukan dalam penelitian ini efisien untuk diterapkan dimana harga komersial PLA2 racun ular *Crotalus amandetus* (Worthington, USA) adalah Rp. 590.000 per mg (59.00 US Dolar).

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Hasil pengolahan data citra satelit tahun (2006) yang diunduh dari website NASA pada Juni 2013 memperlihatkan luas areal terumbu karang yang merupakan habitan *Acanthaster planci* adalah 94,83 hektar. Diperkirakan pada luas areal tersebut terdapat 550 individu dewasa dan jumlah yang dapat dimanfaatkan untuk menghasilkan PLA2 adalah 20% dari ketersediaannya per bulan.

Berdasarkan hasil percobaan tersebut dapat disimpulkan bahwa :

• Metode sederhana dan cepat dengan biaya operasional relatif murah melalui pengendapan 20% amonium sulfat terhadap crude venom yang dipanaskan terlebih dahulu efektif memurnikan PLA2 dari racun duri *Acanthaster planci* dengan tingkat kemurnian dan aktifitas spesifik yang tinggi. Sedangkan metode pengendapan menggunakan etanol 80% tidak efektif memurnikan PLA2 dari racun duri *Acanthaster planci* namun dapat meningkatkan aktivitasnya menjadi 5 kali crude venom. PLA2 racun duri *Acanthaster planci* memiliki aktifitas antibakteri terhadap *Staphylococcus aureus* dan aktifitas antiHIV.

• Biaya miniscale operasional purifikasi PLA2 efisien untuk diterapkan dan ketersediaan *Acanthaster planci* di perairan Liang dan pulau Pombo yang dapat dimanfaatkan untuk menghasilkan PLA2 adalah sebesar 20% per bulan.

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<b>ABSTRACT</b><br>

Spines venom of *Acanthaster planci* have various biological activities: lethal activity, hemolytic, myonecrotic, bleeding, increased capillary permeability, edema, phospholipase A2 (PLA2), the activity of histamine release from mast cells and cardio vascular activity. Spines venom of *Acanthaster planci* containing phospholipase A2 (PLA2), plancitoxin which is homologous with mammals deoxyribonuklease II and plancinin anticoagulant peptide. Previous studies prove that the venoms derived from animals contain various compounds that are potential to be developed as antibiotic and therapeutic agents to treat a disease. *Acanthaster planci* spines venom with various potential biological activity may contribute in the medical field that can be input for the state revenue. Antimicrobial effect results by hydrolysis activity of PLA2 on microbial cell membrane phospholipids can be beneficial to the development of antibiotic agent. PLA2 purified from snake venom have antibacterial activity against *Staphylococcus aureus*, *Proteus vulgaris*, *Proteus mirabilis*, and *Burkholderia pseudomallei*. In addition, PLA2 has antiHIV activity through inhibition of the release mechanism of intracellular viral capsid proteins and assumed PLA2 blocking viral entry into host cells before the virus opens membranes and independently utilize koreseptornya. PLA2 protect human T lymphocytes by blocking viruses that have outer

sheath containing phospholipids.

*Acanthaster planci* is a predator that threatens coral populations, especially when there is an outbreak population. *Acanthaster planci* utilization for the production of PLA<sub>2</sub> can be an effort to control the population productively and make it more useful. Purification of *Acanthaster planci* spines venom PLA<sub>2</sub> has been done by Shiomi and colleagues by using a series of chromatography columns which is relatively expensive and takes a few days, so a simple and fast method with a relatively low cost was developed in this study.

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The results of this study

are expected to be input for utilization of *Acanthaster planci* to produce PLA<sub>2</sub> that can be developed as antibacterial and anti-HIV agents.

Experiments method were conducted in this study gave the following results:

• Venom extraction from the spines of *Acanthaster planci* was effective through the process of sonication at 20 kHz for 2x8 minutes (intensity 80% and 10 outputs).

Venom was accumulated in extraction medium solution of 0.01 M phosphate buffer pH 7.0 containing 0.001 M CaCl<sub>2</sub> called crude venom. Qualitative tested by using human blood treated with crude venom (1: 1) showed the blood anticoagulation by plancinin contained in the venom, proves the extraction process successfully. At the previous conducted on a method of extraction, the spines were blended first and followed by sonicated. To minimize contaminant proteins derived from spines tissue and consider the efficiency, this method was not implemented. Purification of phospholipase A<sub>2</sub> from spines venom of Ambon-Maluku *Acanthaster*

*planci* by using fractionated ammonium sulfate precipitation at 20% saturation of the heated crude venom was done effectively. SDS-PAGE electrophoresis showed PLA<sub>2</sub> isolates has one protein band while the crude venom has four protein bands. PLA<sub>2</sub> isolates has a specific activity 20 times the specific activity of crude venom. Heated the crude venom at 60°C for 30 minutes followed by centrifugation for 30 minutes at 15.000xg and 4°C separated PLA<sub>2</sub> from the other heat sensitive proteins. This method was also implemented to purify PLA<sub>2</sub> spines venom of *Acanthaster planci* from Sorong-Papua, but have not been successful. While PLA<sub>2</sub> purification by using ethanol precipitation at a level of 80% saturation was not effective but increased the specific activity into five times crude venom specific activity. This Experimental results were published in the International Journal of Pharma and Bio Science Vol 2 / issue 2 / Apr-June 2011 and the International Journal of Pharma and Bio Science 2012 Oct; 3 (4) : ( B ) 603-608.

• Investigated of antibacterial activity by using disc diffusion method exhibited clear zone around the disc pre-added PLA<sub>2</sub> on *Staphylococcus aureus* culture, indicated PLA<sub>2</sub> of *Acanthaster planci* spines venom has antibacterial activity against *Staphylococcus aureus*. This experimental result was published in International journal of Pharma and Bio Science 2013 Apr; 4(2) : ( B ) 1-5

• Qualitative investigated of anti-HIV activity by using PBMCs of HIV patient showed a decrease of the DNA protein band intensity in electrophoresis result of RT-PCR RNA

sample of the HIV cultured treated with PLA2. Furthermore, quantitative analysis of the Green Fluorescence Particle results showed the decline significantly from 9.72% into 0.29% in the number of HIV-infected cells by PLA2 treatment, indicated PLA2 of *Acanthaster planci* spines venom has antiHIV activity. This experimental result was published in Asian Pacific Journal of Tropical Medicine(2014) 412-420

• The cost of PLA2 purification was paid for : 1) chemicals and equipment consumables, 2) electricity for the operation of the tools, 3) tools rental and 4) labor. The cost of PLA2 purification was Rp. 446.192,- per 50 grams spines with the results obtained was 4.622 mg PLA2. Miniscale purification costs performed in this study was efficiently implemented which is the commercial prices PLA2 is ± 590,000 rupiahs per mg (59,00 US dolar) (Worthington USA product of snake venom *Crotalus amandetus* PLA2). Thus purification of PLA2 from *Acanthaster planci* spines venom might be have a good prospect to be developed.

• *Acanthaster planci* survey was done on March 2013 in Eastern part Ambon water, especially in Liang (dusun Tanjung and dusun Batu Dua) and Pombo island obtained the average density value of 5.8 adult individuals per hectare. Satellite images (2006) downloaded from NASA website in June 2013 shown coral reefs area as the habitat of *Acanthaster planci* is 94.83 acres. Total estimated of adult *Acanthaster planci* in those area was 550 and the availability number that can be used to produce PLA2 was 20% per month. , Spines venom of *Acanthaster planci* have various biological activities: lethal activity, hemolytic, myonecrotic, bleeding, increased capillary permeability, edema, phospholipase A2 (PLA2), the activity of histamine release from mast cells and cardio vascular activity. Spines venom of *Acanthaster planci* containing phospholipase A2 (PLA2), plancitoxin which is homologous with mammals deoxyribonuklease II and plancinin anticoagulant peptide. Previous studies prove that the venoms derived from animals contain various compounds that are potential to be developed as antibiotic and therapeutic agents to treat a disease. *Acanthaster planci* spines venom with various potential biological activity may contribute in the medical field that can be input for the state revenue. Antimicrobial effect results by hydrolysis activity of PLA2 on microbial cell membrane phospholipids can be beneficial to the development of antibiotic agent. PLA2 purified from snake venom have antibacterial activity against *Staphylococcus aureus*, *Proteus vulgaris*, *Proteus mirabilis*, and *Burkholderia pseudomallei*. In addition, PLA2 has antiHIV activity through inhibition of the release mechanism of intracellular viral capsid proteins and assumed PLA2 blocking viral entry into host cells before the virus opens membranes and independently utilize koreseptornya. PLA2 protect human T lymphocytes by blocking viruses that have outer sheath containing phospholipids.

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