IDENTIFIKASI PEPTIDA HIDROLISAT VENOM ULAR TANAH (*Calloselasma rhodostoma*) HASIL FRAKSINASI PADA pH TINGGI DAN UJI SEBAGAI ANTIBAKTERI

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INTISARI

Telah dilakukan penelitian identifikasi peptida antibakteri hasil fraksinasi pada pH tinggi dari hidrolisat protein venom *Calloselasma rhodostoma*. Tujuan penelitian ini yaitu (I) mendapatkan hidrolisat protein venom ular *Calloselasma rhodostoma* menggunakan enzim tripsin, (II) mengetahui aktivitas antibakteri fraksi peptida aktif dari hidrolisat hasil fraksinasi pada pH tinggi yaitu pH 8 sampai pH 12 yang di elusi menggunakan kolom DSC-SCX SPE (*Strong Cation Exchange*), (III) memahami urutan asam amino peptida antibakteri dari fraksi hidrolisat dengan *Liquid Chromatography-High Resolution Massa Spectroscopy* (LC-HRMS) mengetahui prediksi mekanisme aksi peptida antibakteri dengan teknik in *silico*.

Protein venom ular *Calloselasma rhodostoma* hidrolisis secara enzimatis menggunakan tripsin. Hidrolisat protein difraksinasi menggunakan kolom DSC-SCX SPE (*Strong Cation Exchange*) penukar kation dengan variasi pH untuk pelarut elusi. Uji aktivitas antibakteri dilakukan terhadap bakteri *Escherichia coli* dan *Staphylococcus aureus*, menggunakan metode difusi cakram. Fraksi yang memiliki aktivitas sebagai agen antibakteri dianalisis dengan LC-HRMS. Struktur peptida teridentifikasi penambatan secara *in silico* dengan ligan asli bactenecin.

Hidrolisis protein venom dengan tripsin diperoleh nilai derajat hidrolisis sebesar 80,23%. Fraksi peptida hasil hidrolisat dengan aktivitas antibakteri paling aktif ditunjukan oleh fraksi pH 10 yaitu pada bakteri *Escherichia coli* dan pada bakteri *Staphylococcus aureus* memberikan diameter hambatan masing-masing sebesar 10,5 dan 10,0 mm. Urutan asam amino peptida adalah VGEVKKDPGLLK, VEDLSKR, dan AGKICRIPR yang diprediksi berpotensi sebagai peptida antibakteri

Kata Kunci : Peptida, venom ular *Calloselasma rhodostoma*, tripsin, antibakteri

IDENTIFICATION OF PEPTIDE *Calloselasma rhodostoma* (MALAYAN PIT-VIPER) VENOM HYDROLYSATE FRACTIONATION PRODUCT AT HIGH pH AND ACTIVITY TEST AS ANTIBACTERIAL AGENT

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ABSTRACT

Research to identify antibacterial peptides from trypsin hydrolyzed proteins of *Calloselasma rhodostoma* venom has been conducted. The objectives of this research (I) were to obtain the venom protein hydrolysate of *Calloselasma rhodostoma* using trypsin enzyme, (II) to determine the antibacterial activity of the active peptide fraction from the hydrolysate fractionated at high pH 8 to pH 12 using DSC-SCX SPE (*Strong Cation Exchange*) column elution, (III) to identification the sequence of amino acids the active fraction using *Liquid Chromatography-High Resolution Massa Spectroscopy* (LC-HRMS), and determine the mechanism of action of antibacterial peptides using in silico techniques.

The venom protein of *Calloselasma rhodostoma* was enzymatically hydrolyzed using trypsin. The hydrolysate protein is fractionated using a DSC-SCX SPE (*Strong Cation Exchange*) column with varying pH for elution solvents. Antibacterial activity tests are conducted to predict against *Escherichia coli* and *Staphylococcus aureus* bacteria, using disc diffusion method with inhibition zone results for antibacterial activity. The peptides with antibacterial activity were analyzed by LC-HRMS (*High Resolution Mass Spectrometry)*. The identified peptides were docked in silico with the native bactenecin ligand.

Protein hydrolysis with trypsin gave a degree of hydrolysis value of 80.23%. The most active antibacterial activity peptide fraction from the hydrolysate was shown at pH 10 for against to *Escherichia coli* bacteria, and *Staphylococcus aureus* for bacteria with inhibition zone diameters were 10,5 and 10,0 mm respectively. The potential antibacterial peptides VGEVKKDPGLLK, VEDLSKR, and AGKICRIPR, were predicted.

Keywords: peptides, *Calloselasma rhodostoma* snake venom, trypsin, antibacterial