UJI INHIBISI EKSTRAK KOLAGEN KULIT IKAN SALMON TERHADAP MATRIKS METALOPROTEINASE-9 (MMP-9) SECARA *IN VITRO* DAN *IN SILICO*

SKRIPSI

diajukan untuk memenuhi salah satu syarat memperoleh gelar Sarjana Sains pada Program Studi Kimia

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ABSTRAK

Proses penyembuhan luka dipengaruhi oleh banyak faktor, salah satunya adalah aktivitas Matriks Metaloproteinase-9 (MMP-9). Overekspresi MMP-9 dapat menyebabkan degradasi *extracellular* matriks (ECM) yang lebih tinggi dari biasanya, sehingga memperlambat penutupan luka kulit. Kulit pada produksi ikan merupakan bagian produk samping yang belum banyak dimanfaatkan, padahal kandungan proteinnya yang tinggi dapat menjadi alternatif sumber kolagen untuk sumber inhibitor alami MMP-9. Pada penelitian ini dilakukan pengujian aktivitas inhibisi MMP-9 oleh ekstrak kolagen dari limbah kulit ikan Salmon secara *in vitro* dan *in silico*. Berdasarkan karakterisasi, ekstrak menunjukkan keberadaan kolagen. Hal ini ditandai dengan didapatkannya kadar protein sebesar 95,33 ± 2,20 (%). Selain itu, karakterisasi ekstrak dengan FTIR menunjukkan serapan khas gugus hidroksiprolin dan amida, karakterisasi SDS-PAGE menunjukkan pita rantai alpha, karakterisasi UV VIS menunjukkan puncak khas kolagen, dan karakterisasi XRD menunjukkan sebaran amorf dan jarak asam amino. Pengujian *in vitro* inhibisi enzim MMP-9 menunjukkan persen inhibisi relatif sebesar 71,17%; 76,06%; 82,29%; 90,05%; 72,07%; dan 75,64% pada konsentrasi 10; 100; 250; 500; 1000; dan 2000 ppm. Inhibisi tertinggi diperoleh pada konsentrasi ekstrak kolagen sebesar 500 ppm. Pengujian secara *in silico* menunjukkan afinitas pengikatan kolagen dan MMP-9 sebesar -262,02 kkal/mol dan melibatkan 103 residu asam amino. Prediksi *in silico* peptida aktif hasil hidrolisis enzimatik dengan bromelain, papain, pepsin, tripsin, *chymotrypsin*, dan *thermolysin* menghasilkan tiga peptida aktif dengan afinitas pengikatan terbesar adalah WF (Trp-Phe), YW(Tyr-Trp), dan VW(Val-Trp) dengan nilai afinitas pengikatan masing-masing sebesar -9; -8,6; dan -8,6 kkal/mol. Pengikatan peptida aktif dengan enzim melibatkan interaksi van der Waals, ikatan hidrogen, ikatan hidrofobik, pi-sulfur, dan interaksi *unfavorable*. Inhibisi peptida aktif diprediksi termasuk ke dalam tipe kompetitif. Berdasarkan hasil pengujian *in silico* dan *in vitro*, ditemukan bahwa kolagen dan peptida aktif dari ikan salmon memiliki potensi dalam menghambat enzim MMP-9.

Kata kunci: *Wound healing*, kolagen ikan salmon, peptida aktif, MMP-9

ABSTRACT

The wound healing process is influenced by many factors, one of which is the activity of Matrix Metalloproteinase-9 (MMP-9). Overexpression of MMP-9 can lead to extracellular higher in normal skin. Fish skin is part of the by-product that has not been widely used, even though its high protein content can be an alternative source of collagen for a source of natural inhibitors of MMP-9. In this study, the inhibitory activity of MMP-9 was tested by extracting collagen from salmon skin waste based on in vitro and in silico studies. Based on the characterization, the extract showed the presence of collagen. This is indicated by the obtained protein content of 95.33 ± 2.20 (%). In addition, characterization of extracts with FTIR showed typical absorption of hydroxyproline and amide groups, SDS-PAGE characterization showed alpha chain bands, UV VIS characterization showed typical peaks of collagen, and XRD characterization showed amorphous distribution and amino acid distance. In vitro assay of MMP-9 enzyme inhibition showed a relative inhibition of 71.17%; 76.06%; 82.29%; 90.05%; 72.07%; and 75.64% at concentration 10; 100; 250; 500; 1000; and 2000 ppm. The highest inhibition was obtained at a collagen extract concentration of 500 ppm. Meanwhile, in silico assay showed binding affinity score of -262.02 kcal/mol from MMP-9 and collagen binding and involved 103 amino acid residues. Enzymatic hydrolysis of salmon collagen with bromelain, papain, pepsin, trypsin, chymotrypsin, and thermolysin resulting the three active peptides with the greatest binding affinity were WF (Trp-Phe), YW(Tyr-Trp), and VW(Val-Trp) with binding affinity values of -9; -8.6; and -8.6 kcal/mol. The binding of active peptides with enzymes involves van der Waals interactions, hydrogen bonds, hydrophobic bonds, pi-sulfur, and unfavorable. The active peptide inhibition is predicted as competitive inhibition. Based on the results of in silico and in vitro, it was found that collagen and active peptides from salmon had the potential to inhibit the MMP-9 enzyme.

Keywords: *Wound healing, Salmon collagen, active peptide, MMP-9*

DAFTAR ISI

DAFTAR TABEL

DAFTAR GAMBAR

DAFTAR LAMPIRAN

DAFTAR ISTILAH, SINGKATAN, DAN LAMBANG

A : Alanin (Ala)

MMP-9 : *Matrix metalloproteinase-9*

ASC : *Acid Soluble Collagen*

C : Sistein (Cys)

D : Asam aspartat (Asp)

E : Asam glutamat (Glu)

F : Fenilalanin (Phe)

G : Glisin (Gly)

H : Histidin (His)

HBD : *Hydrogen Bond Donor*

HBA : *Hydrogen Bond Acceptor*

I : Isoleusin (Ile)

K : Lisin (Lys)

L : Leusin (Leu)

M : Metionin (Met)

N : Asparagin (Asn)

P : Prolin (Pro)

PSC : *Pepsin Soluble Collagen*

Q : Glutamin (Gln)

R : Arginin (Arg)

RMSD : *Root Mean Square Deviation*

S : Serin (Ser)

T :Threonin (Thr)

V : Valin (Val)

W : Triptofan (Trp)

Y : Tyrosin (Tyr)

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