ABSTRAK

Prevalensi penyakit inflamasi terus meningkat hingga 20% populasi dunia. Obat anti-inflamasi masih berisiko efek samping, sementara kolagen mengandung asam amino hidrofobik dan bermuatan positif yang dapat menghambat mediator inflamasi. Kolagen cacing sipou (*Siphonosoma australe*) dapat dimanfaatkan sebagai sumber alternatif anti-inflamasi alami. Proses ekstraksi kolagen dan hidrolisis enzimatis dapat menghasilkan peptida bioaktif cacing sipou sebagai anti-inflamasi *inhibitor cyclooxygenase*-2 (COX-2) belum pernah dilaporkan. Penelitian ini bertujuan untuk: 1) mempelajari pengaruh waktu ekstraksi asam asetat terhadap rendemen dan aktivitas anti-inflamasi serta karakteristik kolagen cacing sipou; 2) mempelajari pengaruh waktu pencernaan dan berat molekul (BM) protein hidrolisat kolagen cacing sipou hasil simulasi pencernaan *in vitro* terhadap aktivitas anti-inflamasinya; 3) mempelajari karakteristik peptida cacing sipou dan interaksi peptida *inhibitor* dengan enzim COX-2. Kebaruan dari penelitian ini adalah informasi komprehensif mengenai profil peptida bioaktif anti-inflamasi (*inhibitor* COX-2) dari cacing sipou. Penelitian ini terdiri dari 3 tahap: 1) ekstraksi kolagen cacing sipou; 2) simulasi pencernaan *in vitro* menggunakan enzim pepsin-pankreatin dilanjutkan dengan fraksinasi; 3) karakterisasi dan identifikasi *sequence* peptida bioaktif serta evaluasi interaksi peptida-enzim COX-2 (*in silico*). Parameter uji pada penelitian ini yaitu rendemen, protein, pH, viskositas, komposisi total asam amino, berat molekul (BM), dan gugus fungsi kolagen, serta aktivitas *inhibitor* COX-2 secara *in vitro*, derajat hidrolisis (DH) kolagen, konsentrasi peptida, pola dan klasifikasi penghambatan peptida *inhibitor* COX-2, *sequence* peptida, *molecular docking*, prediksi toksisitas dan bioaktivitas lain peptida. Hasil penelitian ini menunjukkan bahwa ekstraksi kolagen selama 72 jam memiliki nilai rendemen sebesar 3,55±0,20% dan aktivitas *inhibitor* COX-2 sebesar 41,12±0,58%. Kolagen cacing sipou memiliki karakteristik umum kolagen, terlihat pada nilai kadar protein kolagen sebesar 85,58±0,1%, pH kolagen sebesar 3,61, viskositas kolagen sebesar 8-9 cP, komposisi asam amino hidrofobik kolagen sebesar 23,58% dan bermuatan positif sebesar 16,23%, BM kolagen rantai α 123 kDa dan rantai β 280 kDa, serta analisis gugus fungsi memperlihatkan adanya struktur *triple helix* kolagen pada bilangan gelombang 1239 cm^{-1}. Hidrolisis *in vitro* memperlihatkan DH sebesar 84,03%, konsentrasi peptida sebesar 1,12 mg/mL dan menghasilkan fraksi peptida <1 kDa dengan aktivitas *inhibitor* COX-2 tertinggi sebesar 89,05%. Peptida bioaktif cacing sipou memiliki pola penghambatan kompetitif dan klasifikasi peptida *substrate type*. Identifikasi *sequence* peptida fraksi <1 kDa ditemukan tiga peptida anti-inflamasi baru, yaitu ADIAGQAAQVLR, LNNEITTLR dan VGTVEK, dengan BM 631-1,212 Da dan didominasi oleh asam amino hidrofobik pada terminal-N dan asam amino bermuatan positif pada terminal-C. *Molecular docking* memperlihatkan interaksi terjadi pada sisi aktif enzim COX-2 melalui interaksi hidrofobik, ikatan hidrogen, interaksi van der Waals dan interaksi elektrostatik. Hasil prediksi toksisitas menunjukkan bahwa semua peptida cacing sipou bersifat non toksik dan memiliki potensi bioaktivitas lain, seperti ACE, DPP-IV dan α-glucosidase *inhibitor* secara *in silico*.

Kata kunci: Cacing sipou, *inhibitor* COX-2, *in-silico*, kolagen, *sequence* peptida, simulasi pencernaan *in vitro*

ABSTRAK

The prevalence of inflammatory diseases continues to rise, affecting up to 20% of the global population. Anti-inflammatory drugs still pose risks of side effects, whereas collagen contains hydrophobic and positively charged amino acids that can inhibit inflammatory mediators. Peanut worm (*Siphonosoma australe*) collagen has the potential to be utilized as a natural alternative anti-inflammatory source. However, the extraction of collagen and enzymatic hydrolysis to produce bioactive peptides from peanut worm as cyclooxygenase-2 (COX-2) inhibitors has not been reported. This study aims to: (1) investigate the effect of acetic acid extraction time on yield, anti-inflammatory activity, and the characteristics of peanut worm collagen; (2) investigate the effect of digestion time and molecular weight (MW) of collagen hydrolysate derived from the peanut worm through in vitro digestion simulation on its anti-inflammatory activity; (3) characterize peanut worm peptides and analyze peptide-inhibitor interactions with the COX-2 enzyme. The novelty of this research lies in providing comprehensive information on the bioactive anti-inflammatory peptide profile (COX-2 inhibitors) from peanut worms. This study consists of three stages: (1) extraction of peanut worm collagen; (2) *in vitro* digestion simulation using pepsin-pancreatin enzymes followed by fractionation; and (3) characterization and identification of bioactive peptide sequences, along with the evaluation of peptide-enzyme COX-2 interactions (*in silico*). The parameters assessed in this study include yield, protein content, pH, viscosity, total amino acid composition, molecular weight (MW), collagen functional groups, *in vitro* COX-2 inhibition activity, degree of hydrolysis (DH), peptide concentration, COX-2 inhibitor peptide patterns and classification, peptide sequences, molecular docking, and predictions of peptide toxicity and bioactivity. The results indicate that collagen extraction for 72 h yielded 3.55±0.20% with a COX-2 inhibition activity of 41.12±0.58%. Peanut worm collagen exhibits typical collagen characteristics, with a protein content of 85.58±0.1%, pH of 3.61, viscosity of 8–9 cP, hydrophobic amino acid composition of 23.58%, and positively charged amino acids at 16.23%. The MW of α and β-chain collagen are 123 and 280 kDa, with functional group analysis confirming a triple-helix collagen structure at a wavenumber of 1239 cm^{-1}. *In vitro* hydrolysis resulted in a DH of 84.03%, a peptide concentration of 1.12 mg/mL, and the production of peptide fractions <1 kDa, which exhibited the highest COX-2 inhibition activity at 89.05%. The bioactive peptides from peanut worms demonstrated a competitive inhibition pattern and were classified as substrate-type peptides. Peptide sequence identification of the <1 kDa fraction revealed three novel anti-inflammatory peptides: ADIAGQAAQVLR, LNNEITTLR, and VGTVEK, with MWs ranging from 631 to 1,212 Da, predominantly featuring hydrophobic amino acids at the N-terminal and positively charged amino acids at the C-terminal. Molecular docking analysis showed interactions at the active site of the COX-2 enzyme through hydrophobic interactions, hydrogen bonds, van der Waals forces, and electrostatic interactions. Toxicity predictions indicated that all peanut worm peptides were non-toxic and possessed additional potential bioactivities, such as ACE, DPP-IV, and α-glucosidase inhibition, as determined *in silico*.

Keywords: Peanut worm, COX-2 inhibitor, *in silico*, collagen, peptide sequence, *in vitro* digestion simulation.