

INTISARI

Permasalahan hiperpigmentasi kulit telah mendorong pengembangan agen pencerah berbasis bahan alam, salah satunya berasal dari alga coklat *Padina australis* Hauck yang diketahui mengandung senyawa aktif fukosantin. Penelitian ini bertujuan untuk mengevaluasi aktivitas biologis ekstrak, fraksi etil asetat (FEA), dan isolat fukosantin dari *Padina australis* Hauck dalam menghambat enzim tirosinase dan kolagenase, menilai pengaruhnya terhadap viabilitas sel terhadap sel vero dan fibroblas, serta mengkaji potensi penghambatan proliferasi dan pembentukan melanin pada sel melanoma B16F10.

Proses ekstraksi dilakukan menggunakan pelarut etanol, diikuti fraksinasi dengan etil asetat. Isolasi senyawa aktif dilakukan menggunakan kromatografi lapis tipis preparatif (KLTP) dan menghasilkan fukosantin sebagai senyawa utama. Evaluasi aktivitas biologis sampel (ekstrak, fraksi, isolat fukosantin dan standar fukosantin) dilakukan melalui beberapa tahapan pengujian secara *in-vitro*. Uji aktivitas penghambatan enzim tirosinase dan kolagenase dilakukan untuk mengetahui potensi sampel sebagai agen antipigmentasi dan antikolagenase. Uji viabilitas sel dilakukan menggunakan metode MTT terhadap sel vero dan sel fibroblas untuk menilai tingkat toksisitas terhadap sel normal. Aktivitas antiproliferatif terhadap sel melanoma B16F10 dianalisis guna menentukan nilai IC_{50} . Selain itu, uji depigmentasi dilakukan pada kultur sel B16F10 yang diinduksi dengan α -melanocyte stimulating hormone (α -MSH) untuk mengevaluasi kemampuan fraksi dalam menurunkan sintesis melanin. Stabilitas fisikokimia sediaan emulgel yang mengandung FEA diuji selama 30 hari melalui pengukuran parameter daya lekat, daya sebar, viskositas, dan nilai *sun protection factor* (SPF).

Hasil penelitian menunjukkan bahwa isolat fukosantin dari *Padina australis* dengan kemurnian sebesar 96,8% memiliki aktivitas penghambatan 50% (IC_{50}) tirosinase tertinggi kojic acid ($9,43 \pm 4,86 \mu\text{g/mL}$), diikuti oleh FEA ($33,47 \pm 3,33 \mu\text{g/mL}$), FTEA ($45,80 \pm 8,06 \mu\text{g/mL}$), ekstrak ($49,48 \pm 0,85 \mu\text{g/mL}$), isolat fukosantin ($89,78 \pm 3,87 \mu\text{g/mL}$), standar fukosantin senilai $273,4 \pm 1,65$. Sedangkan aktivitas penghambatan kolagenase pada kadar $100 \mu\text{g/mL}$, tertinggi ditunjukkan oleh FEA ($94,92 \pm 2,94\%$), isolat fukosantin dan ekstrak ($94,02 \pm 0,34\%$). Viabilitas sel tertinggi terhadap sel vero ditemukan pada ekstrak ($4.145 \pm 51 \mu\text{g/mL}$), sedangkan terhadap sel fibroblas tertinggi pada isolat fukosantin ($11.493 \pm 11 \mu\text{g/mL}$). FEA dan ekstrak menunjukkan aktivitas antiproliferatif terhadap sel melanoma B16F10 yang tidak berbeda signifikan (IC_{50} 224 ± 12 dan $229 \pm 19 \mu\text{g/mL}$), sedangkan isolat fukosantin menunjukkan aktivitas lebih tinggi (IC_{50} $200 \pm 18 \mu\text{g/mL}$). Nilai depigmentasi tertinggi diperoleh dari FEA ($53 \pm 2,2\%$), diikuti dengan ekstrak ($47,7 \pm 6,2$) dan isolat fukosantin ($30,3 \pm 7,06$). Isolat fukosantin memiliki nilai penghambatan tirosinase yang lebih tinggi tetapi secara statistik tidak berbeda bermakna dengan FEA. Nilai FEA untuk kolagenase dan depigmentasi juga lebih tinggi daripada isolat fukosantin sehingga untuk pembuatan emulgel digunakan FEA. Hasil formulasi emulgel berbasis FEA menunjukkan stabilitas fisikokimia yang baik selama penyimpanan serta mempunyai nilai SPF sebesar $13,289 \pm 0,185$. Berdasarkan hasil tersebut, fraksi etil asetat dari *Padina australis* Hauck berpotensi sebagai bahan aktif alami untuk formulasi kosmetik pencerah kulit yang efektif dan aplikatif secara farmasetik.

Kata kunci: *Padina australis* Hauck, isolat fukosantin, tirosinase, kolagenase, depigmentasi

ABSTRACT

The issue of skin hyperpigmentation has driven the development of natural-based brightening agents, one of which is derived from the brown algae *Padina australis* Hauck, known to contain the active compound fucoxanthin. This study aims to evaluate the biological activity of the extract, ethyl acetate fraction (EAF), and fucoxanthin isolate from *Padina australis* Hauck in inhibiting tyrosinase and collagenase enzymes, assess their effects on the viability of Vero and fibroblast cells, and investigate their potential to inhibit proliferation and melanin formation in B16F10 melanoma cells.

The extraction process was carried out using ethanol, followed by fractionation with ethyl acetate. The active compound was isolated using preparative thin-layer chromatography (PTLC), resulting in fucoxanthin as the main compound. The biological activities of the samples (extract, fraction, fucoxanthin isolate, and standard fucoxanthin) were evaluated through several in-vitro tests. Tyrosinase and collagenase enzyme inhibition assays were conducted to assess the potential of the samples as anti-pigmentation and anti-collagenase agents. Cell viability tests were performed using the MTT method on Vero and fibroblast cells to evaluate toxicity on normal cells. The antiproliferative activity on B16F10 melanoma cells was analyzed to determine the IC_{50} values. Additionally, a depigmentation test was conducted on B16F10 cell cultures induced with α -melanocyte stimulating hormone (α -MSH) to evaluate the ability of the fraction to reduce melanin synthesis. The physicochemical stability of an emulgel formulation containing the EAF was tested over 30 days by measuring parameters such as adhesion, spreadability, viscosity, and sun protection factor (SPF).

The results showed that the fucoxanthin isolate from *Padina australis* with a purity of 96.8% had the highest tyrosinase inhibitory activity (IC_{50}) after kojic acid ($9.43 \pm 4.86 \mu\text{g/mL}$), followed by EAF ($33.47 \pm 3.33 \mu\text{g/mL}$), FTEA ($45.80 \pm 8.06 \mu\text{g/mL}$), extract ($49.48 \pm 0.85 \mu\text{g/mL}$), fucoxanthin isolate ($89.78 \pm 3.87 \mu\text{g/mL}$), and standard fucoxanthin ($273.4 \pm 1.65 \mu\text{g/mL}$). For collagenase inhibition at a concentration of $100 \mu\text{g/mL}$, the highest activity was observed with EAF ($94.92 \pm 2.94\%$), followed by fucoxanthin isolate and extract ($94.02 \pm 0.34\%$). The highest cell viability for Vero cells was found with the extract ($4,145 \pm 51 \mu\text{g/mL}$), while for fibroblast cells, it was with the fucoxanthin isolate ($11,493 \pm 11 \mu\text{g/mL}$). EAF and extract showed comparable antiproliferative activity against B16F10 melanoma cells (IC_{50} values of 224 ± 12 and $229 \pm 19 \mu\text{g/mL}$, respectively), whereas the fucoxanthin isolate exhibited higher activity ($IC_{50} = 200 \pm 18 \mu\text{g/mL}$). The highest depigmentation value was obtained from EAF ($53 \pm 2.2\%$), followed by extract ($47.7 \pm 6.2\%$) and fucoxanthin isolate ($30.3 \pm 7.06\%$). Although the fucoxanthin isolate had higher tyrosinase inhibition, it was not significantly different from EAF. EAF also showed higher collagenase inhibition and depigmentation activity compared to the fucoxanthin isolate; therefore, EAF was selected for the emulgel formulation. The EAF-based emulgel formulation demonstrated good physicochemical stability during storage and had an SPF value of 13.289 ± 0.185 . Based on these results, the ethyl acetate fraction of *Padina australis* Hauck has potential as a natural active ingredient for the formulation of effective and pharmaceutically applicable skin-brightening cosmetics.

Keywords: *Padina australis*, fucoxanthin isolate, tyrosinase, collagenase, depigmentation