UNIVERSITAS GADJAH MADA
AKTIVITAS EKSTRAK ETANOL DAUN KITOLOD (Isotoma longiflora (L.) C. Presl) DALAM PENYEMBUHAN ULKUS DIABETIK MELALUI MEKANISME ANTIINFLAMASI DAN PROLIFERASI SEL: Studi Terintegrasi In Silico, In Vitro, dan In Vivo
Happy Elda Murdiana, Prof. Dr. apt. Zullies Ikawati
Universitas Gadjah Mada, 2026 | Diunduh dari http://etd.repository.ugm.ac.id/

INTISARI

Ulkus diabetes ditandai oleh inflamasi persisten yang menghambat transisi ke fase proliferasi dan menyebabkan kegagalan regenerasi jaringan. Penekanan inflamasi secara dini menjadi prasyarat untuk mengaktifkan proliferasi dan angiogenesis dalam penyembuhan ulkus. Penelitian ini bertujuan untuk mengungkap mekanisme molekuler ekstrak etanol daun kitolod (EEDK) dalam mempercepat penutupan ulkus Diabetes Mellitus (DM) melalui pendekatan terintegrasi *in silico, in vitro, dan in vivo.*

Penelusuran kandungan fitokimia EEDK dilakukan menggunakan metode LC-HRMS dan penetapan kadar lobelin dilakukan menggunakan KLT densitometri. Analisis *in silico* dilakukan melalui *network pharmacology* untuk menentukan target protein kunci dan *molecular docking* untuk mengevaluasi afinitas ikatan. Uji in vitro menggunakan sel HaCaT pada kondisi hiperglikemia dengan parameter proliferasi serta migrasi sel pada 2 4 dan 48 jam, pengukuran kadar TNF-α, IL-6, VEGF-A, TGF-β, MMP-9, dan TIMP-1 (ELISA) serta ekspresi AKT-1, HIF-1α, dan VEGF-A (*Western blot*) dilakukan pada jam ke-6 setelah perlakuan untuk menangkap respons molekuler fase awal. Uji *in vivo* dilakukan pada tikus DM terinduksi streptozotocin (STZ) untuk mengevaluasi kecepatan penutupan ulkus, penurunan jumlah sel inflamasi, kecepatan re-epitelisasi, dan deposisi kolagen.

Hasil penelitian menunjukkan EEDK mengandung senyawa lobelin, asam kafeat,asam siringat dan asam ferulat yang berpotensi sebagai penyembuh luka DM. Lobelin merupakan senyawa dominan dengan kadar 0,947% dalam ekstrak dan 0,0023% dalam simplisia. Analisis *in silico* mengidentifikasi AKT-1, MAPK3, dan HIF-1α sebagai protein kunci, dengan afinitas ikatan lobelin terhadap AKT-1 lebih rendah dibandingkan vildagliptin dan sitagliptin. Secara in vitro, EEDK meningkatkan proliferasi secara signifikan pada seluruh konsentrasi (125-0,98ppm) ($p<0,0001$), dan juga lobelin 1,95 ppm ($p=0,0491$). Migrasi sel meningkat signifikan pada 24 jam (EEDK 15,62 ppm, $p=0,0022$; lobelin 1,95 ppm, $p=0,0009$) dan 48 jam (EEDK 15,62 ppm, $p=0,0006$; lobelin 1,95 ppm, $p=0,0005$). Pada pengamatan jam ke-6, EEDK menurunkan TNF-α (31,25 ppm, $p=0,0050$), sementara lobelin meningkatkan VEGF-A (3,90 ppm, $p=0,0486$); MMP-9 juga meningkat signifikan pada EEDK 15,62 ppm ($p=0,0431$) dan lobelin 1,95 ppm ($p=0,0003$). Rasio MMP-9/TIMP-1 lobeline 3,90 ppm meningkat signifikan ($p=0,0095$) dibanding kontrol glukosa tinggi, disertai peningkatan ekspresi AKT-1, HIF-1α, dan VEGF-A oleh EEDK. Secara in vivo, EEDK mempercepat penutupan ulkus tergantung dosis dan waktu, menurunkan infiltrasi sel inflamasi, mempercepat re-epitelisasi pada hari ke-14, dan menghasilkan ketebalan epitel mendekati normal tanpa menimbulkan jaringan parut abnormal, serta terjadi deposisi kolagen lengkap pada hari ke-21. Temuan ini menegaskan EEDK bekerja melalui penekanan inflamasi awal yang diikuti aktivasi proliferasi, angiogenesis, dan remodeling, serta berpotensi sebagai terapi topikal ulkus diabetes.

Kata kunci: ulkus diabetik, anti-inflamasi, angiogenesis, migrasi sel, sel HaCaT, re-epitelisasi, deposisi kolagen

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ABSTRACT

Diabetic ulcers are characterized by persistent inflammation that inhibits the transition to the proliferative phase and leads to tissue regeneration failure. Early suppression of inflammation is a prerequisite for activating proliferation and angiogenesis in ulcer healing. This study aims to uncover the molecular mechanism of ethanolic extract of kitolod leaves (EEDK) in accelerating the closure of diabetic ulcers through an integrated in silico, in vitro, and in vivo approach.

The phytochemical content of EEDK was investigated using the LC-HRMS method, and lobeline levels were determined using TLC densitometry. In silico analysis was performed using network pharmacology to identify key protein targets and molecular docking to evaluate binding affinity. In vitro tests using HaCaT cells under hyperglycemic conditions, with cell proliferation and migration parameters measured at 24 and 48 hours. TNF-α, IL-6, VEGF-A, TGF-β, MMP-9, and TIMP-1 levels were measured (ELISA), and AKT-1, HIF-1α, and VEGF-A expression (Western blot) were measured at 6 hours after treatment to capture the early phase of molecular responses. In vivo tests were conducted on streptozotocin (STZ)-induced diabetic rats to evaluate the rate of ulcer closure, reduction in inflammatory cell count, re-epithelialization rate, and collagen deposition.

The results showed that EEDK contains lobeline, caffeic acid, syringic acid, and ferulic acid, which have potential as diabetic wound healing agents. Lobeline was the dominant compound, with concentrations of 0.947% in the extract and 0.0023% in the crude drug. In silico analysis identified AKT-1, MAPK3, and HIF-1α as key proteins, with lobeline having a lower binding affinity for AKT-1 than vildagliptin and sitagliptin. In vitro, EEDK significantly increased proliferation at all concentrations (125-0.98 ppm) ($p<0.0001$), as well as at 1.95 ppm of lobeline ($p=0.0491$). Cell migration was significantly increased at 24 hours (EEDK 15.62 ppm, $p=0.0022$; lobeline 1.95 ppm, $p=0.0009$) and 48 hours (EEDK 15.62 ppm, $p=0.0006$; lobeline 1.95 ppm, $p=0.0005$). At 6 hours of observation, EEDK decreased TNF-α (31.25 ppm, $p=0.0050$), while lobeline increased VEGF-A (3.90 ppm, $p=0.0486$); MMP-9 was also significantly increased at EEDK 15.62 ppm ($p=0.0431$) and lobeline 1.95 ppm ($p=0.0003$). The MMP-9/TIMP-1 ratio of lobeline 3.90 ppm increased significantly ($p=0.0095$) compared to the high glucose control, accompanied by increased expression of AKT-1, HIF-1α, and VEGF-A by EEDK. In vivo, EEDK accelerated ulcer closure in a dose- and time-dependent manner, reduced inflammatory cell infiltration, accelerated re-epithelialization on day 14, and resulted in near-normal epithelial thickness without abnormal scarring, and complete collagen deposition occurred on day 21. These findings confirm that EEDK works through suppression of early inflammation followed by activation of proliferation, angiogenesis, and remodeling, and has potential as a topical therapy for diabetic ulcers.

Keywords: diabetic ulcer, antiinflammatory, angiogenesis, cells migration, HaCaT cell, re-epithelization, collagen deposition