

Efek Fraksi Aktif *Piper crocatum* Ruiz & Pav terhadap Deposisi Kolagen dan Wound Closure melalui Regulasi Ekspresi p53, α SMA, SOD1 dan E-cadherin pada Wounded Hyperglycemia Fibroblasts

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ABSTRAK

Latar belakang: *Piper crocatum* Ruiz & Pav (*P. crocatum*) telah dilaporkan dapat mempercepat penyembuhan luka diabetik secara empiris. Beberapa studi menunjukkan manfaat *P. crocatum* dalam mengobati berbagai macam penyakit termasuk luka kronik. *P. crocatum* dilaporkan dapat menginduksi proliferasi fibroblas, namun studi lain melaporkan *P. crocatum* menghambat proliferasi. Oleh karena itu, pemisahan senyawa pada *P. crocatum* perlu dilakukan untuk mendapatkan efek yang konsisten.

Tujuan penelitian: Penelitian ini bertujuan untuk mengidentifikasi efek fraksi aktif *P. crocatum* terhadap aktivitas *in vitro* pada wounded hyperglycemia fibroblasts (wHFs).

Metode Penelitian: Penelitian ini merupakan penelitian *in vitro* dengan menggunakan fibroblas hiperglikemia (HFs) yang ditumbuhkan dari kultur primer jaringan sirkumsisi pada media basal dengan penambahan 17 mM/L *D-glucose*. Identifikasi fraksi aktif dilakukan menggunakan prosedur *Bioassay-guided fractionation* dan didasarkan pada nilai IC50 tertinggi. Fraksi paling aktif kemudian diaplikasikan pada HFs yang dilukai dengan *diagonal scratch* (wHFs). Deposisi kolagen diukur dengan menggunakan *Picro-Sirius Red* dan wound closure diukur dengan menggunakan *scratch assay*. Ekspresi p53, α SMA, SOD1 dan E-cadherin diukur menggunakan *western blotting*.

Hasil Penelitian: Penelitian ini mengkonfirmasi bahwa fraksi IV (F_{IV}) adalah fraksi aktif *P. crocatum*. Kelompok wHFs intervensi memiliki rerata deposisi kolagen ($p < 0.0001$) dan wound closure rate ($p < 0.0001$) lebih tinggi secara signifikan dibandingkan kelompok kontrol. Kelompok intervensi juga ditemukan memiliki ekspresi p53 lebih rendah secara signifikan dibandingkan dengan kelompok kontrol ($p = 0.001$). Ekspresi α SMA, SOD1 dan E-cadherin ditemukan lebih tinggi pada kelompok intervensi daripada kelompok kontrol ($p = 0.0001$). Hasil uji korelasi menemukan deposisi kolagen dan wound closure berhubungan negatif dengan ekspresi p53 ($p = 0.01$, $r = -1.000$), dan berhubungan positif dengan α SMA, SOD1 dan E-cadherin ($p = 0.01$, $r = 1.000$). Profil KLT menunjukkan adanya kandungan senyawa polifenol glikosida.

Kesimpulan: F_{IV} *P. crocatum* berpotensi dapat mempercepat penyembuhan luka pada wHFs pada konsentrasi paling efektif 7.81 μ g/ml dengan meningkatkan deposisi kolagen dan kecepatan wound closure melalui penurunan p53 serta peningkatan α SMA, SOD1, dan E-cadherin. Golongan senyawa polifenol glikosid diduga berkontribusi pada mekanisme tersebut.

Kata Kunci: *Piper crocatum* Ruiz & Pav; penyembuhan luka diabetik; mekanisme; p53; α SMA; SOD1; E-cadherin.

ABSTRACT

Background: *Piper crocatum* Ruiz & Pav (*P. crocatum*) has been reported to accelerate the diabetic wound healing process empirically. Some studies showed the benefits of *P. crocatum* in treating various diseases. The effects of *P. crocatum* on fibroblasts have been reported to induce proliferation and vice versa. Hence, the separation of *P. crocatum* compounds needs to be done to get a consistent effect on diabetic wound healing.

Aims: This study aimed to identify the effects of *P. crocatum* active fractions on *in vitro* activities of wounded hyperglycemia fibroblasts (wHFs).

Methods: This study was an *in vitro* study using hyperglycemia fibroblasts (HFs) cultured from circumcision tissues and grown on basal media with the addition of 17mMol/L D-glucose. Active fraction was identified following bioassay-guided fractionation procedure and was determined based on the highest IC₅₀ values. The most active fraction then was applied on wHFs which was diagonal scratched. Collagen deposition were measured using Picro-Sirius Red and wound closure were measured using scratch assay. The expressions of p53, α SMA, SOD1, and E-cadherin were measured using western blotting and counted using Image J for Windows. The compound of F_{IV} was determined using thin layer chromatography (TLC).

Results: This study confirmed that fraction IV (F_{IV}) is the most active fraction of *P. crocatum*. Further findings, the intervention group had significantly higher collagen deposition rates ($p < 0.0001$) and wound closure rates ($p < 0.0001$) than the control group. The treatment group was also found to have a significantly lower p53 relative expression than the control group ($p = 0.001$). The expressions of α SMA, SOD1 and E-cadherin were found to be higher in the intervention group than the control group ($p = 0.0001$). The correlation tests showed a negative relationship between collagen deposition and wound closure with expressions of p53 ($p = 0.001$, $r = -1.000$), and a positive relationship with α SMA, SOD1, and E-cadherin ($p = 0.001$, $r = 1.000$) at F_{IV} of 7.81 μ g/dl. The TLC profile showed the presence of glycoside polyphenols.

Conclusion: F_{IV} *P. crocatum* has the potential to accelerate wound healing in wHFs by increasing the rates of collagen deposition and wound closure. Regulations in p53 as well as α SMA, SOD1, and E-cadherin are known to underlie these events at 7.81 μ g/dl the most optimal concentration of F_{IV}. The glycoside polyphenols are supposed to contribute to these mechanisms.

Key words: *Piper crocatum* Ruiz & Pav; diabetic wound healing; mechanism; p53; α SMA; SOD-1; E-cadherin.