Bioassay guided Isolation Senyawa Aktif Herba Ciplukan (*Physalis angulata* L.) Termonitor Glucose Consumption Assay: Isolasi dan Identifikasi, Molecular docking, dan Penelusuran Mekanisme Aksinya Pada Sel Myoblast

Rita Rakhmawati
21/484489/SKU/873

ABSTRAK

Latar belakang: Herba ciplukan adalah salah satu tanaman yang berpotensi untuk dikembangkan sebagai obat diabetes. Penelitian sebelumnya menunjukkan fraksi 1 herba ciplukan paling aktif dalam meningkatkan konsumsi glukosa pada sel C2C12. Penelitian ini bertujuan menelusuri senyawa aktif dan mekanisme aksi isolat aktif herba ciplukan terhadap ekspresi protein fosforilasi IRS-1^{Tyr612} dan GLUT-4 pada sel C2C12 resisten insulin, serta mekanisme molekuler melalui *molecular docking*.

Metode: Pelaksanaan fraksinasi menggunakan kromatografi vakum cair (VLC), isolasi menggunakan KLT preparatif, dan pemantauan dengan KLT serta uji konsumsi glukosa pada sel C2C12. Identifikasi senyawa menggunakan GC-MS, FTIR, HPLC, ^{1}H NMR, ^{13}C NMR, DEPT, HSQC, dan HMBC. Pengujian sitotoksisitas menggunakan MTT *assay*, penilaian konsumsi glukosa menggunakan pengukuran kadar glukosa medium dengan GOD-PAP, dan penilaian ekspresi protein GLUT-4 serta p-IRS1^{Tyr612} menggunakan western blot. Sel C2C12 terinduksi asam palmitat (PA 0,75 mM) merupakan model resistensi insulin. Kelompok perlakuan diberikan PA serta isolat pada berbagai konsentrasi (1/8, 1/4, dan 1/2 IC_{50}). Uji *in silico* menggunakan *Autodock Tools* 4.2. Data dianalisis dengan Oneway ANOVA dan Post Hoc Tukey atau Kruskal-Wallis dan Mann-Whitney dengan signifikansi nilai $p < 0,05$.

Hasil: Penelitian ini menghasilkan senyawa isolat berupa serbuk kristal putih. Spektrum FTIR menunjukkan gugus OH (3432 cm^{-1}), C=C (1624 cm^{-1}), dan C-O (1057 cm^{-1}). Kromatogram HPLC menunjukkan satu puncak dengan dua puncak berdekatan. Hasil GC-MS menunjukkan campesterol (m/z 400) dan stigmasterol (m/z 412). Spektrum ^{1}H NMR menunjukkan proton alkana dan alkena. Spektrum HMBC menunjukkan korelasi proton-karbon pada kerangka steroid. Senyawa yang diidentifikasi adalah fitosterol campuran stigmasterol dan campesterol (Phy CamStig). Perlakuan dengan fitosterol (Phy CamStig) menghasilkan kadar glukosa medium sel C2C12 *myotube* resisten insulin yang lebih rendah, dan ekspresi GLUT-4 membran yang lebih tinggi, namun ekspresi p-IRS1^{Tyr612} setara dengan kontrol. Phy CamStig tidak toksik pada C2C12 *myotube* dengan IC_{50} 210 µg/mL.

Kesimpulan: Senyawa hasil isolasi teridentifikasi sebagai fitosterol campuran campesterol dan stigmasterol (Phy Camstig). Pemberian Phy CamStig memperbaiki konsumsi glukosa sel C2C12 *myotube* resisten insulin, dan ekspresi GLUT-4, namun tidak mempengaruhi ekspresi p-IRS1$^{Tyr612.}$ Simulasi *in silico* menunjukkan Phy CamStig berpotensi dalam pengobatan diabetes tipe 2 dengan menargetkan GLUT-4 melalui fasilitasi translokasi GLUT-4 dan memiliki afinitas tinggi pada situs pengikatan 1 pada reseptor IRS-1.

Kata Kunci: *Physalis angulata*, Campesterol, Stigmasterol, GLUT-4, IRS-1, docking

Bioassay Guided Isolation of Active Compounds from *Physalis angulata* L. Herb Monitored by Glucose Consumption Assay: Isolation and Identification, Molecular Docking, and Mechanistic Investigation in Myoblast Cells

Rita Rakhmawati
21/484489/SKU/873

ABSTRACT

Background: Ciplukan herb is one of the plants that has the potential to be developed as a diabetes drug. Previous research showed that fraction 1 of the herb was most active in increasing glucose consumption in C2C12 cells. This study aims to investigate the active compounds and the mechanism of action of active isolates of herbs on the expression of IRS-1Tyr612 and GLUT-4 phosphorylated proteins in insulin-resistant C2C12 cells, as well as molecular mechanisms through *molecular docking*.

Methods: Implementing fractionation using liquid vacuum chromatography (VLC), isolation using preparative KLT, and monitoring with KLT and glucose consumption test in C2C12 cells. The compound was identified using GC-MS, FTIR, HPLC, ^{1}H NMR, ^{13}C NMR, DEPT, HSQC, and HMBC. Cytotoxicity testing was performed using MTT *assay*, glucose consumption assessment using medium glucose level measurement with GOD-PAP, GLUT-4 protein expression assessment, and p-IRS1Tyr612 using western blot. Palmitic acid-induced C2C12 cells (PA 0.75 mM) are a model of insulin resistance. The treatment group was given PA and isolated at various concentrations (1/8, 1/4, and 1/2 IC_{50}). Test *in silico* using *Autodock Tools* 4.2. The data were analyzed with Oneway ANOVA and Post Hoc Tukey or Kruskal-Wallis and Mann-Whitney with a significance $p <$ value of 0.05.

Results: This research produced an isolated compound in the form of white crystalline powder. The FTIR spectrum shows the OH (3432 cm^{-1}), C=C (1624 cm^{-1}), and C-O (1057 cm^{-1}) groups. HPLC chromatograms show one peak with two peaks close together. The GC-MS spectrum shows campesterol (m/z 400) and stigmasterol (m/z 412). The NMR ^{1}H spectrum shows alkane and alkene protons. The HMBC spectrum shows a proton-carbon correlation in the steroid skeleton. The compounds identified were a mixture of stigmasterol and campesterol (Phy CamStig). Treatment with phytosterols (Phy CamStig) resulted in lower glucose levels of insulin-resistant C2C12 *myotube* cell medium and higher expression of membrane GLUT-4, but equivalent expression of p-IRS1Tyr612 to control. Phy CamStig is non-toxic in C2C12 *myotubes* with IC_{50} 210 µg/mL.

Conclusion: The isolated compounds were identified as a mixture of campesterol and stigmasterol (Phy Camstig). Administration of Phy CamStig improved glucose consumption of insulin-resistant myotube C2C12 cells and GLUT-4 expression but did not affect p-IRS1Tyr612 expression. In silico *simulations* show Phy CamStig has potential in treating type 2 diabetes by targeting GLUT-4 through GLUT-4 translocation facilitation and having a high affinity for binding site one on the IRS-1 receptor.

Keywords: *Physalis angulata*, Campesterol, Stigmasterol, GLUT-4, IRS-1, docking