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Aktivitas Anti Kanker Piper crocatum Ruiz & Pav, Piper retrofractum Vahl dan Piper aduncum L Terhadap Cell line T47D: Kajian Seluler, Molekuler, dan Insilico
Jantje Wiliem Souhaly, Prof. Dra. Rarastoeti Pratiwi, M.Sc. Ph.D; Prof. Dr. Laurentius Hartanto Nugroho, M.Agr; Dr. T
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INTISARI

Kanker payudara merupakan penyebab utama kematian akibat kanker pada wanita dengan prevalensi yang terus meningkat. Penggunaan kemoterapi seringkali terkendala oleh resistensi dan toksisitas pada sel normal, sehingga diperlukan kandidat ko-kemoterapi baru berbasis bahan alam. Genus *Piper* dikenal memiliki potensi sitotoksik, namun perbandingan efektivitas dan mekanisme molekuler spesifik dari *Piper crocatum* Ruiz & Pav, *Piper retrofractum* Vahl, dan *Piper aduncum* L belum dikaji secara komprehensif. Penelitian ini bertujuan untuk menapis aktivitas sitotoksik ketiga spesies *Piper*, mencari fraksi yang paling potensial dari ekstrak terpilih terhadap sel T47D dan Vero, serta menganalisis mekanisme aksinya melalui penghambatan migrasi, induksi apoptosis, siklus sel, ekspresi gen, dan prediksi interaksi molekuler secara *in silico*. Penelitian ini menggunakan ekstrak daun *P. crocatum*, *P. retrofractum*, dan *P. aduncum* dengan pelarut etanol. Sampel didapatkan dari Merapi Farma, Yogyakarta. Ekstrak etanol ketiga tanaman diuji sitotoksisitasnya menggunakan metode MTT pada sel kanker payudara T47D dan sel Vero. Ekstrak yang terpilih difraksinasi dengan metode *VLC* dengan pelarut campuran dari n-heksan, etil asetat, metanol dengan perbandingan volume tertentu dan kromatografi lapis tipis (KLT). Uji sitotoksik dilakukan untuk memperoleh kandidat fraksi yang paling toksik terhadap sel T47D dan tidak toksik pada sel Vero. Fraksi terpilih diuji mekanismenya menggunakan *scratch wound healing assay*, *flow cytometry* (*Annexin V-PI*), dan qPCR (*Caspase-3*, *p21*). Identifikasi senyawa fraksi dilakukan dengan *LC-HRMS* dan diprediksi interaksinya melalui *molecular docking* dan *molecular dynamics simulation*. Hasil penelitian menunjukkan ekstrak etanol daun *P. crocatum* memiliki nilai IC_{50} yang rendah terhadap sel Vero (207,38 µg/mL) dibandingkan ekstrak lainnya dan terhadap sel T47D termasuk dalam kategori toksisitas sedang (IC_{50} 129,84). Hasil VLC menghasilkan 9 fraksi yang di KLT menjadi 4 fraksi. Fraksi aktif FIII memiliki sitotoksisitas tinggi terhadap T47D (IC_{50} 47,22 µg/mL) dan selektif terhadap sel Vero (SI 2,81). Secara mekanistik, fraksi FIII mampu menghambat migrasi sel, menginduksi apoptosis, dan menahan siklus sel pada fase G0/G1. Analisis qPCR menunjukkan peningkatan ekspresi gen *Caspase-3* sebagai eksekutor apoptosis. Hasil Fraksi FIII dengan LC-HRMS dan *in silico* mengidentifikasi senyawa *3-hydroxyl-2',5-dimethoxy-2-methylbibenzyl* sebagai kandidat senyawa dengan afinitas pengikatan yang stabil terhadap protein Bcl-2 dan CDK1. Fraksi FIII daun *Piper crocatum* cukup berpotensi dikembangkan

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sebagai kandidat agen antikanker payudara yang efektif dan selektif melalui mekanisme antimetastasis, induksi apoptosis jalur *Caspase-3* dan penghambatan siklus sel.

Kata kunci: Genus *Piper*, Sel T47D, Antikanker, Apoptosis, Siklus Sel, Metastasis, *In silico*

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Anticancer Activity of *Piper crocatum* Ruiz & Pav, *Piper retrofractum* Vahl, and *Piper aduncum* L Against T47D *Cell line:* Study Cellular, Molecular and *In silico*

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ABSTRACT

Breast cancer is the leading cause of cancer-related mortality among women, with an increasing prevalence worldwide. The application of chemotherapy is often limited by resistance and toxicity to normal cells, thereby necessitating the development of novel co-chemotherapeutic agents derived from natural compounds. The Piper genus is known for its cytotoxic potential; however, the comparative effectiveness and specific molecular mechanisms of *Piper crocatum* Ruiz & Pav, *Piper retrofractum* Vahl, and *Piper aduncum* L have not been comprehensively studied. This research aims to screen the cytotoxic activities of these three Piper species, identify the most potent fraction from the selected extract against T47D and Vero cells, and elucidate its mechanism of action through migration inhibition, apoptosis induction, cell cycle arrest, gene expression analysis, and *in silico* molecular interaction prediction. This study utilized ethanolic extracts of the leaves of *P. crocatum, P. retrofractum,* and *P. aduncum*, obtained from Merapi Farma, Yogyakarta. The cytotoxicity of the ethanolic extracts was assessed using the MTT assay on T47D breast cancer cells and Vero cells. The selected extract was fractionated by vacuum liquid chromatography (VLC) using solvent mixtures of n-hexane, ethyl acetate, and methanol in specific volume ratios, and analyzed by thin-layer chromatography (TLC). Cytotoxic testing was conducted to identify the most active fraction that exhibited toxicity toward T47D cells but minimal toxicity to Vero cells. The mechanism of action of the selected fraction was further evaluated using scratch wound healing assays, flow cytometry (Annexin V-PI), and qPCR targeting *Caspase-3* and *p21*. Fraction identification compound was performed using LC-HRMS, followed by molecular docking and molecular dynamics simulation for interaction prediction. The results demonstrated that the ethanolic extract of *P. crocatum* leaves exhibited a relatively low IC_{50} value against Vero cells (207.38 µg/mL) compared to the other extracts and showed moderate toxicity against T47D cells (IC_{50} 129,84). VLC fractionation yielded nine fractions, which were grouped into four fractions based on TLC profiles. The active fraction, FIII, showed high cytotoxicity against T47D cells (IC_{50} = 47.22 µg/mL) and selectivity toward Vero cells (SI = 2.81). Mechanistically, fraction FIII inhibited cell migration, induced apoptosis, and caused cell cycle arrest at the G0/G1 phase. qPCR analysis revealed an upregulation of *Caspase-3* expression as an apoptosis executor, although *p21* expression changes were not significant. LC-HRMS and *in silico* analyses identified 3-hydroxyl-2',5-dimethoxy-2-methylbibenzyl as the main candidate compound with stable binding affinity to its

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target receptor Bcl-2 and CDK1. In conclusion, fraction FIII from *Piper crocatum* leaves shows potential as a selective and effective breast cancer therapeutic candidate through mechanisms of antimetastatic, apoptosis induction via the *Caspase-3* pathway and cell cycle inhibition.

Keywords: Genus *Piper*, T47D cell line, Anti-cancer, Apoptosis, Cell cycle, Metastasis, *In silico*