DISERTASI

KAJIAN AKTIVITAS SITOTOKSIK EKSTRAK ETANOL DAUN *Garcinia cowa* Roxb. SEBAGAI AGEN KO-KEMOTERAPI TRASTUZUMAB PADA SEL KANKER PAYUDARA MCF-7/HER2

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ABSTRAK

Kajian Aktivitas Sitotoksik Ekstrak Etanol Daun *Garcinia cowa* Roxb. sebagai Agen Ko-Kemoterapi Trastuzumab pada Sel Kanker Payudara MCF-7/HER2

Kanker payudara HER2-positif bersifat agresif, sering mengalami resistensi terhadap trastuzumab, serta menimbulkan efek samping kardiotoksik. Penelitian ini bertujuan menilai interaksi garcinisidone-A (Gar-A) dengan reseptor HER2 secara *in silico* dan menguji sinergisme ekstrak etanol daun *Garcinia cowa* (EEDGC) dengan trastuzumab pada sel kanker payudara MCF-7/HER2 secara *in vitro*.

Untuk aspek *in silico*, struktur kristal HER2 (PDB 3PP0) dipersiapkan di UCSF Chimera, sedangkan Gar-A dioptimasi dan di-*docking* menggunakan GNINA. Pose terbaik dianalisis di *Discovery Studio Visualizer*, dan prediksi ADME Gar-A diperoleh dari aplikasi pkCSM. Simulasi dinamika molekuler 100 ns dijalankan di GROMACS (CHARMM36) untuk mengevaluasi stabilitas kompleks Gar-A-HER2. Metode *in vitro* meliputi penentuan sitotoksisitas EEDGC dan trastuzumab, baik secara tunggal maupun kombinasi pada sel MCF-7/HER2 dan sel normal vero melalui uji MTT. Nilai IC_{50} diperoleh untuk masing-masing perlakuan, kemudian dilakukan perhitungan Indeks Kombinasi (IK) menggunakan metode Chou-Talalay untuk menilai tingkat sinergisme. Distribusi fase siklus sel dan induksi apoptosis dianalisis dengan *flow cytometry*. Selain itu, ekspresi protein regulator siklus sel (p53, siklin D1, dan siklin E) diukur menggunakan antibodi berkonjugasi fluorokrom.

Secara *in silico*, Gar-A memiliki berat molekul <500 Da, logP=2,8, kelarutan air sedang, permeabilitas usus baik, tidak mudah menembus sawar darah-otak, serta klirens cepat melalui rute ekskresi atau metabolisme enzim. Nilai skor afinitas CNN pada reseptor HER2 sebesar 6,946 menunjukkan potensi interaksi yang mirip ligan alami. Gar-A membentuk ikatan hidrogen dengan residu Lys753 dan Asp863, ikatan karbon-hidrogen dengan Leu785, Ser783, Thr862, serta ikatan alkil dengan Leu726, Leu852, dan Ile767. Hasil MD 100 ns menampilkan fluktuasi RMSD < 0,2 nm setelah 5 ns dan RMSF rendah pada residu kunci, meski kestabilan kompleks sedikit menurun menjelang akhir simulasi. Hasil *in vitro* menunjukkan bahwa EEDGC memiliki IC_{50} sebesar 119,21 µg/mL, sedangkan trastuzumab sebesar 954,52 µg/mL, EEDGC memiliki indeks selektivitas terhadap sel vero sebesar 2,55 Kombinasi optimal 25 µg/mL EEDGC (¼ IC_{50}) dan 125 µg/mL trastuzumab (⅛ IC_{50}) menghasilkan efek sinergi yang kuat (Fa=0,897; IK=0,266, DRI Trastuzumab > 1, DRI EEDGC >1), memicu hambatan fase G_2-M dan G_0-G_1, menurunkan viabilitas sel menjadi 73,4%, dan meningkatkan proses apoptotik sel sebesar 17,2%. Analisis protein siklus sel memperlihatkan peningkatan ekspresi p53 serta penurunan siklin D1 sehingga mendukung mekanisme hambatan proliferasi.

Secara keseluruhan, Gar-A menunjukkan prediksi karakteristik farmakokinetik dan interaksi molekuler yang memenuhi aturan Lipinski serta potensi antikanker secara *in silico*. EEDGC meningkatkan efektivitas trastuzumab

melalui modulasi siklus sel dan induksi apoptosis, dengan selektivitas yang baik terhadap sel kanker dibandingkan sel normal. Temuan ini mendukung pengembangan EEDGC sebagai terapi kombinasi trastuzumab dan Gar-A sebagai calon molekul terapi baru untuk kanker payudara HER2-positif. Studi lanjutan *in vivo* dan validasi klinis diperlukan untuk mengonfirmasi keamanan dan efikasi dalam konteks organisme utuh.

ABSTRACT

Cytotoxic Activity Study of Ethanolic Extract of *Garcinia cowa* Roxb. Leaves as a Co-Chemotherapy Agent to Trastuzumab in MCF-7/HER2 Breast Cancer

HER2-positive breast cancer is highly aggressive, frequently develops resistance to trastuzumab, and is associated with cardiotoxic side effects. This study aimed to assess the molecular interaction of garcinisidone-A (Gar-A) with the HER2 receptor *in silico* and to evaluate the synergistic effect of *Garcinia cowa* ethanol leaf extract (EEDGC) combined with trastuzumab on MCF-7/HER2 breast cancer cells *in vitro*.

For the *in silico* study, the crystal structure of HER2 (PDB ID: 3PP0) was prepared using UCSF Chimera, while Gar-A was geometrically optimized and subjected to molecular docking using GNINA. The best docking pose was analyzed in Discovery Studio Visualizer, and the pharmacokinetic properties (ADME) of Gar-A were predicted using the pkCSM platform. A 100-nanosecond molecular dynamics (MD) simulation was conducted in GROMACS with the CHARMM36 force field to evaluate the stability of the Gar-A-HER2 complex. The *in vitro* assays included cytotoxicity evaluation of EEDGC and trastuzumab, both individually and in combination, on MCF-7/HER2 cancer cells and normal Vero cells using the MTT assay. IC_{50} values were determined for each treatment, followed by calculation of the Combination Index (CI) using the Chou-Talalay method to assess synergy. Cell-cycle phase distribution and apoptosis induction were analyzed using flow cytometry. Additionally, the expression levels of key cell-cycle regulatory proteins (p53, cyclin D1, and cyclin E) were quantified using fluorochrome-conjugated antibodies.

In silico analysis revealed that Gar-A possesses a molecular weight below 500 Da, logP of 2.8, moderate water solubility, good intestinal permeability, limited blood-brain barrier penetration, and rapid clearance via metabolic or excretory pathways. The CNN affinity score of 6.946 indicated a binding potential comparable to natural ligands. Gar-A formed hydrogen bonds with Lys753 and Asp863, carbon-hydrogen interactions with Leu785, Ser783, and Thr862, and alkyl interactions with Leu726, Leu852, and Ile767. MD simulation for 100 ns showed RMSD fluctuations below 0.2 nm after 5 ns and low RMSF values at key residues, despite slight reductions in complex stability towards the end of the simulation. *In vitro* results demonstrated that EEDGC exhibited an IC_{50} of 119.21 µg/mL, while trastuzumab had an IC_{50} of 954.52 µg/mL. EEDGC showed a selectivity index (SI) of 2.55 against cancer cells relative to normal vero cells. The optimal combination of 25 µg/mL EEDGC (¼ IC_{50}) and 125 µg/mL trastuzumab (⅛ IC_{50}) produced a strong synergistic effect (Fa=0.897; CI=0.266, DRI Trastuzumab > 1, DRI EEDGC >1), inducing cell-cycle arrest at the G_2-M phase and G_0-G_1 phase, reducing cell viability to 73.4%, and enhancing apoptosis to 17.2%. Cell-cycle protein analysis showed an increase in p53 expression and a decrease in cyclin D1, supporting the mechanism of proliferation inhibition.

Overall, Gar-A demonstrated favorable pharmacokinetic predictions, compliance with Lipinski's rule of five, and significant molecular interaction potential with HER2 *in silico*. EEDGC enhanced the therapeutic efficacy of trastuzumab by modulating the cell cycle and inducing apoptosis, with good selectivity toward cancer cells over normal cells. These findings support the potential development of EEDGC as a co-chemotherapeutic agent alongside trastuzumab and suggest Gar-A as a promising candidate for novel targeted therapy in HER2-positive breast cancer. Further in vivo studies and clinical validation are warranted to confirm safety and efficacy in a whole-organism context.