

Aktivitas Antikanker Kolorektal dan Imunomodulator Minyak Kulit Ikan Layang Biru terhadap Sel WiDr dan RAW264.7: Studi *In Vitro* dan *In Silico*

INTISARI

Pengobatan kanker kolorektal dengan mengandalkan kemoterapi doksorubisin menimbulkan toksisitas dan immunosupresif. Kulit ikan layang biru yang mengandung beragam asam lemak berpotensi sebagai agen antikanker kolorektal dan imunomodulator. Penelitian ini bertujuan untuk menguji efek antikanker minyak layang biru (MLB) melalui uji sitotoksitas sel WiDr dan sel epitel ginjal (Vero) dengan MTT *assay*, efek penghambatan progresi siklus sel dan kematian sel dengan *flow cytometry*. Penelitian ini juga mengkaji efek imunomodulator MLB melalui uji viabilitas pada sel makrofag RAW264.7, uji aktivitas fagositosis dengan *latex beads* dan *neutral red*, serta uji produksi oksida nitrit dengan *Griess assay*. Analisis prediksi gen target secara *in silico* dilakukan terhadap *eicosapentaenoic acid* (EPA) dan *docosahexaenoic acid* (DHA) yang berperan pada persinyalan antikanker dan imunomodulasi. MLB menghasilkan rendemen sebesar $14,80 \pm 0,28\%$ dengan kadar air 0,52%, abu 0,13%, lemak 90,38%, protein 7,15%, dan karbohidrat 1,82%. Nilai kestabilan oksidatif MLB memenuhi standar IFOS meliputi asam lemak bebas 1,15%, angka peroksida 6,51 meq/kg, p-Anisidin 9,07, dan TOTOX 22,09. Asam lemak dominan dari MLB meliputi asam palmitat, metil oleat, EPA, dan DHA. MLB menunjukkan sitotoksitas lemah ($IC_{50} = 955,76 \mu\text{g/mL}$) terhadap sel WiDr namun tidak bersifat toksik terhadap sel Vero ($IC_{50} = 2.865,78 \mu\text{g/mL}$). MLB menghambat progresi siklus sel WiDr di fase G2/M dan memicu apoptosis pada fase awal dengan konsentrasi 1 dan $\frac{1}{2} IC_{50}$. MLB tidak toksik terhadap sel RAW264.7 dengan viabilitas >93%, mampu meningkatkan aktivitas fagositosis dan menaikkan produksi oksida nitrit pada konsentrasi 31,25–1.000 $\mu\text{g/mL}$. Analisis *in silico* menunjukkan DHA dan EPA menargetkan gen *STAT3*, *P53*, *BCL2*, *RARA*, *RARB* yang berperan dalam mekanisme regulasi siklus sel dan apoptosis kanker. EPA dan DHA juga menargetkan gen *PPARG*, *MMP9*, *IKBKB*, dan *MAPK* dalam respons imun. Temuan ini menunjukkan bahwa MLB potensial untuk dikembangkan sebagai agen antikanker dan imunomodulator.

Kata kunci: Antikanker; Imunomodulator; Kanker kolorektal; Kulit ikan layang biru; Minyak.

Anticancer Activity of Mackerel Scad Skin Oil against WiDr and RAW264.7 Cells: *In Vitro* and *In Silico* Studies

ABSTRACT

Treatment of colorectal cancer with doxorubicin chemotherapy is associated with toxicity and immunosuppression. Mackerel scad skin contains various fatty acids hold potential as both anticancer and immunomodulatory agents. This study aims to evaluate the anticancer effects of mackerel scad oil (MLB) through cytotoxicity assays on WiDr colorectal cancer cells and renal epithelial cells (Vero) using the MTT assays, along with analysis of cell cycle arrest and apoptosis via flow cytometry. The immunomodulatory effects of MLB were also assessed using viability assays on RAW264.7 macrophage cells, phagocytosis tests with latex beads and neutral red, and nitric oxide production using the Griess method. *In silico* target gene prediction analysis was performed on eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), two major fatty acids involved in anticancer signaling and immunomodulation. MLB yielded $14.80 \pm 0.28\%$, with a moisture of 0.52%, ash 0.13%, fat 90.38%, protein 7.15%, and carbohydrates 1.82%. The oxidative stability parameters of MLB met IFOS standards, including free fatty acids 1.15%, peroxide value 6.51 meq/kg, p-anisidine value 9.07, and TOTOX value 22.09. Major fatty acids identified included palmitic acid, methyl oleate, EPA, and DHA. MLB exhibited weak cytotoxicity against WiDr cells ($IC_{50} = 955.76 \mu\text{g/mL}$) and was non-toxic to Vero cells ($IC_{50} = 2,865.78 \mu\text{g/mL}$). MLB inhibited WiDr cell cycle progression at the G2/M phase and triggered early apoptosis at $\frac{1}{2}$ and 1 IC_{50} concentrations. MLB was also non-toxic to RAW264.7 cells with viability $>93\%$ and enhanced phagocytosis activity and nitric oxide production at concentrations ranging from 31.25 – 1,000 $\mu\text{g/mL}$. *In silico* analysis revealed that DHA and EPA target genes such as *STAT3*, *TP53*, *BCL2*, *RARA*, and *RARB*, which are involved in cell cycle regulation and apoptosis in cancer. Furthermore, EPA and DHA were found to target *PPARG*, *MMP9*, *IKBKB*, and *MAPK* which are associated with immune responses. These findings suggest that MLB has promising potential as an agent for cancer therapy and immune modulation.

Keywords: Anticancer; Colorectal cancer; Immunomodulator; Mackerel scad skin; Oil.