ABSTRACT

Malaria as a *Plasmodium* infectious disease still has drug resistance problem and a need for new drug discovery. *Streptomyces* bacteria are a major source of antibiotics. Indonesian *Streptomyces* bacteria from marine (GMY01), mangroves (SHP 22-7 and BSE7F), and forest soil (GMR22) exhibited anticancer, antibacterial, and antifungal activity but study on its antiplasmodial activity were still limited. The study aimed to identify potential active metabolites as antiplasmodial and anticancer of selected *Streptomyces* based on whole-genome analysis, metabolite profiling, and molecular docking.

Genome mining was performed using AntiSMASH version 5 and BiG-FAM tools. Metabolite profiling was conducted using untargeted LC-MS/MS and targeted LC-HRMS. Bioassay-guided fractionation was conducted using flash and column chromatography combined with *in vitro* antiplasmodial on *Plasmodium falciparum* using microscopic and SYBR Green I method and *in vitro* anticancer on several cell lines. A toxicity assay was conducted on the Vero cell line. Gene clusters encoding active metabolites were identified using RAST and The SEED Viewer version 2. *In silico* molecular docking was performed on 5 target proteins of *P. falciparum* and 4 target proteins of cancer cells.

Genome mining analysis revealed that four selected Indonesian *Streptomyces* bacteria have NRPS, PKS, and terpene as dominant biosynthesis gene clusters (BGCs). The majority of the *Streptomyces* produce compounds containing CHON elements with MW approximately 100-400 Da. Marine bacterium *Streptomyces* sp. GMY01 has the highest antiplasmodial activity with low toxicity on normal cells. Three active metabolites were obtained from GMY01, namely F2 and F4.7 from the ethyl acetate extract of supernatant and EMB-B from the methanol extract of cell biomass. Based on the selectivity index, the F2, F4.7, and EMB-B fractions were classified as active, moderate, and active as antiplasmodial, respectively. Meanwhile, F2, F4.7, and EMB-B fractions were categorized as moderate, low active, and active as anticancer, respectively. The major compounds detected in F2 fraction were gabazine ($C_{15}H_{17}N_3O_3$), toyocamycin ($C_{12}H_{13}N_5O_4$), and 4-n-pentanoylbiphenyl ($C_{17}H_{18}O$); in F4.7 fraction were isobutyranilide ($C_{10}H_{13}NO$), propoxur ($C_{11}H_{15}NO_3$) and ephedrine ($C_{10}H_{15}NO$) and in EMB-B fraction was as N-acetylneuraminyl-(2-6)-galactose ($C_{17}H_{29}N_{14}$) and mannotriose ($C_{18}H_{32}O_{16}$). Targeted LC-HRMS analysis showed that albaflavenone ($C_{15}H_{22}O$) was confirmed as an active compound produced by *Streptomyces* sp. GMY01. BGCs encoding albaflavenone was terpene type with 100% deduced amino acids similarity. The KEEG map analysis showed that GMY01 has gene clusters encoding biosynthesis of galactose and mannose, terpene backbone, and alkaloids. Molecular docking analysis showed that albaflavenone and mannotriose have a binding affinity on glutathione reductase (GR) and glutathione-S-transferase (GST) of *P. falciparum* and on autophagy proteins (mTORC1 and mTORC2) of cancer cells. This indicated that these compounds were potential for the antiplasmodial and anticancer drug candidate.

Keywords: antiplasmodial, anticancer, drug discovery, genome mining, metabolite profiling, *Streptomyces*

ABSTRAK

Malaria sebagai penyakit infeksi *Plasmodium* masih menghadapi permasalahan resistensi obat dan memerlukan penemuan obat baru. Bakteri *Streptomyces* adalah sumber utama senyawa antibiotik. *Streptomyces* asal Indonesia dari laut (GMY01), mangrove (SHP 22-7 dan BSE7F), dan tanah (GMR22) memiliki aktivitas antikanker, antibakteri, dan antijamur tetapi belum diketahui aktivitas antiplasmodium-nya. Penelitian ini bertujuan untuk mengidentifikasi potensi metabolit aktif sebagai antiplasmodium dan antikanker dari *Streptomyces* terpilih berbasis analisis *whole-genome*, analisis profil metabolit, dan *molecular docking*.

Genome mining dilakukan dengan menggunakan AntiSMASH versi 5 dan BiG-FAM. Analisis profil metabolit dilakukan dengan *untargeted* LC-MS/MS dan *targeted* LC-HRMS. *Bioassay-guided fractionation* dilakukan dengan *flash chromatography* dan kolom kromatografi dengan uji *in vitro* antiplasmodium pada *Plasmodium falciparum* menggunakan metode mikroskopis dan SYBR Green I dan uji *in vitro* antikanker pada beberapa lini sel. Uji toksisitas dilakukan pada lini sel Vero. Klaster gen yang mengkode metabolit aktif diidentifikasi dengan RAST dan The SEED Viewer versi 2. *In silico molecular docking* dilakukan pada 5 protein target *P. falciparum* dan 4 protein target sel kanker.

Analisis *genome mining* menunjukkan bahwa empat bakteri *Streptomyces* memiliki NRPS, PKS, dan terpen sebagai klaster gen dominan. Sebagian besar *Streptomyces* menghasilkan senyawa mengandung unsur CHON dengan BM antara 100-400 Da. Bakteri laut *Streptomyces* sp. GMY01 memiliki aktivitas antiplasmodium tertinggi dengan toksisitas rendah pada sel normal. Tiga metabolit aktif didapatkan dari GMY01, yaitu fraksi F2 dan F4.7 dari ekstrak etil asetat supernatan dan fraksi EMB-B dari ekstrak metanol biomassa sel. Berdasarkan indek selektivitasnya, F2, F4.7, dan EMB-B dikategorikan berturut – turut sebagai fraksi aktif, moderat, dan aktif sebagai antiplasmodium dan moderat, aktif rendah dan aktif sebagai antikanker. Senyawa dominan pada fraksi F2 adalah *gabazine* ($C_{15}H_{17}N_3O_3$), *toyocamycin* ($C_{12}H_{13}N_5O_4$), dan *4-n-pentanoylbiphenyl* ($C_{17}H_{18}O$); pada fraksi F4.7 adalah *isobutyranilide* ($C_{10}H_{13}NO$), *propoxur* ($C_{11}H_{15}NO_3$) dan *ephedrine* ($C_{10}H_{15}NO$) dan pada fraksi EMB-B adalah *N-acetylneuraminyl-(2-6)-galactose* ($C_{17}H_{29}N_{14}$) dan *mannotriose* ($C_{18}H_{32}O_{16}$). Analysis *targeted* LC-HRMS menunjukkan *albaflavenone* ($C_{15}H_{22}O$) terkonfirmasi sebagai senyawa aktif yang dihasilkan oleh *Streptomyces* sp. GMY01. Klaster gen yang mengkode *albaflavenone* adalah terpen dengan similaritas asam amino 100%. Analisis KEEG menunjukan GMY01 memiliki kluster gen yang mengkode biosintesis *galactose* dan *mannose*, terpen, dan alkaloid. Analisis *molecular docking* menunjukkan *albaflavenone* dan *mannotriose* memiliki *binding affinity* pada protein glutathione reductase (GR) dan glutathione-S-transferase (GST) *P. falciparum* dan protein autofagi (mTORC1 dan mTORC2) pada sel kanker. Hal ini mengindikasikan bahwa senyawa tersebut berpotensi sebagai kandidat obat antiplasmodium dan antikanker.

Kata kunci: antiplasmodium, antikanker, *drug discovery*, *genome mining*, analisis profil metabolit, *Streptomyces*