ABSTRAK

Malaria merupakan penyakit yang disebabkan oleh parasit plasmodium, yang menjadi masalah besar bagi kesehatan dunia. Telah banyak dilaporkan munculnya strain Plasmodium yang resisten terhadap obat-obat antimalaria yang ada sehingga perlu adanya eksplorasi obat antimalaria baru. Penelitian ini secara umum bertujuan untuk mengeksplorasi fungi endofit tanaman *Artemisia annua* L. sebagai penghasil senyawa antiplasmodium.

Tanaman *A. annua* yang akan digunakan dalam penelitian ini diambil dari $B_2P_2TO_2T$, Tawangmangu. Isolasi fungi endofit dilakukan dengan menggunakan media *Potato Dextrose Agar*. Ekstrak etilasetat media fermentasi selanjutnya dilakukan skrining aktivitas antimalaria menggunakan metode *Heme Polymerization Inhibition Assay*. Fungi endofit yang mempunyai aktivitas inhibitor polimerisasi hem terkuat selanjutnya difermentasi dalam 4 jenis media yang berbeda untuk menentukan media yang sesuai. Senyawa aktif antimalaria diisolasi menggunakan metode *Bioassay Guided Fractionation* dengan menggunakan aktivitas inhibitor polimerisasi hem sebagai penuntunnya. Struktur senyawa aktif diidentifikasi menggunakan LC-MS dan NMR. Aktivitas antiplasmodium isolat dilakukan secara *in vitro* menggunakan metode kultur *Plasmodium falciparum* FCR-3.

Dari penelitian ini telah berhasil diisolasi 7 jenis fungi endofit yang diberi kode IP-1, IP-2, IP-3, IP-4, IP-5, IP-6 dan IP-7. Untuk fungi IP-7 tidak dapat diteliti lebih lanjut karena fungi ini tidak dapat dikultur ulang. Keenam fungi tersebut menunjukkan aktivitas sebagai inhibitor polimerisasi hem dengan nilai IC_{50} masing-masing 7,56±1,29; 2,29±0,68; 8,97±2,40; 7,15±1,42; 10,37±1,04; dan 1,81±0,21 mg/mL. Fungi IP-2 dipilih sebagai obyek penelitian selanjutnya karena memiliki potensi yang sama dengan klorokuin dalam menghambat polimerisasi hem dan mudah untuk dikultur ulang. Dalam media PDB, fungi IP-2 menunjukkan laju pertumbuhan yang paling baik dengan nilai μ 0,019 g/jam dan persen penghambatan polimerisasi hem yang paling besar. Hasil identifikasi baik secara konvensional maupun genetika, menunjukkan bahwa fungi IP-2 mempunyai hubungan kekerabatan yang dekat dengan *Penicillium namyslowskii*. Dari *bioassay guided isolation* diperoleh isolat aktif yang mempunyai aktivitas sebagai antiplasmodium dengan nilai IC_{50} 40,67 ± 9,89 μg/mL. Senyawa dominan dalam isolat tersebut merupakan senyawa golongan triterpenoid.

Kata kunci: endofit *Artemisia annua*, inhibitor polimerisasi hem, antiplasmodium, *Plamodium falciparum* FCR-3, triterpenoid

ABSTRACT

Malaria is a disease that becomes a major problem for the health. WHO reported that approximately 250 million cases of malaria cause 1-3 million deaths for each year. The failure of malaria treatment often caused by how quickly the growth of strains resistant to existing drugs. Therefore, it is necessary to explore new antimalarial drugs to combat this phenomenon. One source that has not been utilized optimally in the exploration of the drug is endophytic microbes. This research generally aims to explore the endophytic fungi of *Artemisia annua* L. as a producer antiplasmodium compound.

A. annua plants that will be used in this study were taken from $B_2P_2TO_2T$, Tawangmangu. Isolation of endophytic fungi performed using Potato Dextrose Agar and Sabouraud Dextrose Agar. Endophytic fungi were recultured to obtain pure cultures. Each pure culture of endophytic fungus was fermented in shaking culture, with the temperature of incubation at room temperature, agitation speed of 250 rpm. The obtained ethyl acetate extract was screened for Heme Polymerization Inhibition activity. The selected fungus was fermented in four different media to determine the metabolite production and appropriate time for metabolite harvesting. Antiplasmodium active compound was isolated using fractionation bioassay guided by hem polymerization inhibitor as guidance. Fractionation process was performed using vacuum liquid chromatography with a mobile phase gradient of n-hexane and ethylacetate. The chemical structure of the active compound was identified using LC-MS and NMR. This compound was tested *in vitro* antiplasmodium activity using continuous culture of *Plasmodium falciparum* FCR-3.

It has been isolated seven endophytic fungi encoded IP-1, IP-2, IP-3, IP-4, IP-5, IP-6 and IP-7. Fungus IP-7 could not be investigated further. The sixth fungi showed activity as a hem polymerization inhibitor with IC_{50} values 7.56 ± 1.29; 2.29 ± 0.68; 8.97 ± 2.40; 7.15 ± 1.42; 10.37 ± 1.04; and 1.81 ± 0.21 mg/mL respectively. Chloroquine as a positive control had IC_{50} value of 2.56 ± 0.47 mg/mL. Fungus IP-2 is selected then as the object of further research because it had the same potential to chloroquine in inhibit the polymerization of hem and very easy to reculture in PDA medium. In four different media, fungus IP-2 showed the most rapid growth in M1 (PDB) and M3 media (containing glucose 3 g/L, malt extract 3 g/L, bactopeptone 0.3 g/L, yeast extract 0.15 g/L, KCl 0.075 g/L, MgSO4. 7 H_2O 0.15 g/L, and KH_2PO_4 0.15 g/L) with growth rate (µ) value of 0.019 g/hour. Nevertheless, in M2 medium (SDB), IP-2 showed the slowest growth with the rate of 0.013 g/hour. PDB medium also gave the best result for greatest quantities of active metabolite production and reached the highest yield on the 7th day of fermentation. Morphology identification using microscope and Genetic analysis suggested fungus IP-2 had a close phylogenetic relationship with *Penicillium namyslowskii*. Bioassay guided isolation was obtained successfully 2 isolates i.e isolate 1 and 2, which demonstrated antiplasmodial activity with IC_{50} values 40.67 ± 9.89 and > 100 µg/mL respectively. The major compound in the active isolate (isolate 1) was triterpenoid.

Key words: endophytic fungi, *Artemisia annua*, inhibitor hem polymerization, antiplasmodial, *Plamodium falciparum* FCR-3, triterpenoid