RINGKASAN

ISOLASI DAN UJI AKTIVITAS ANTIMALARIA EKSTRAK METANOL, FRAKSI DAN ISOLAT-ISOLAT DARI KULIT BATANG CEMPEDAK (*Artocarpus champeden*, Spreng.)

Kulit batang cempedak (*A. champeden*) telah digunakan secara tradisional sebagai obat antimalaria. Untuk dapat dikembangkan menjadi obat antimalaria, sebagai langkah awal, perlu dilakukan isolasi terhadap zat yang aktif sebagai antimalaria yang terkandung di dalamnya.

Penelitian ini dimaksudkan untuk mendapatkan zat aktif antimalaria melalui cara isolasi yang dituntun dengan uji aktivitas antimalaria pada setiap tingkatan pemisahan ekstrak kulit batang *A. champeden.* Di dalam penelitian ini telah dilakukan ekstraksi secara maserasi terhadap serbuk kulit batang *A. champeden* secara berturut-turut menggunakan heksana, diklorometana dan metanol masing-masing selama 1 x 3 x 24 jam.

Ekstrak metanol kemudian diuji aktivitas antimalarianya terhadap kultur non-sinkronisasi *Plasmodium falciparum* strain-3D7 secara *in-vitro* selama masa inkubasi 48 jam. Hasil pengujian ini menunjukkan bahwa ekstrak metanol memiliki aktivitas sebagai antimalaria, dengan nilai IC_{50} sebesar : 4,230 ± 1,147 µg/mL. Ekstrak metanol selanjutnya difraksinasi secara kromatografi kolom. Sebanyak 12 gram ekstrak metanol difraksinasi dalam kolom yang berisi *slurry* silika gel sebanyak 400 gram, dengan eluen $CHCl_3$-EtOAc (1:9), $CHCl_3$-MeOH (9:1), $CHCl_3$-MeOH (8:2), $CHCl_3$-MeOH (1:1), dan MeOH. Fraksi-fraksi yang diperoleh selanjutnya digabung-gabungkan berdasarkan kesamaan atau kemiripan profil Kromatografi Lapis Tipis (KLT), dan dihasilkan 8 (delapan) fraksi utama. Masing-masing fraksi diuji lagi aktivitas antimalarianya. Fraksi kedua (M.II) menunjukkan aktivitas paling tinggi dalam menghambat pertumbuhan *Plasmodium*, dengan nilai IC_{50} sebesar 0,146 ±

0,046 µg/mL, dan karena itu, fraksi M.II ini dipilih untuk dipisahkan lebih lanjut (diisolasi).

Fraksi M.II kemudian dipisahkan lebih lanjut secara KLT preparatif, dan dihasilkan 8 (delapan) subfraksi. Dari sub-subfraksi M.II.4, M.II.5 dan M.II.6 diperoleh masing-masing 1 (satu) isolat dalam bentuk padatan amorf, yang selanjutnya dinamai sebagai isolat M.II.4.1, M.II.5.1 dan M.II.6.1. Isolat-isolat ini kemudian diuji aktivitas antimalarianya. Hasil pengujian aktivitas antimalaria isolat-isolat terhadap kultur *P. falciparum* selama masa inkubasi 48 jam memberikan nilai IC_{50} untuk masing-masing isolat sebesar : 0,052 ± 0,011 µg/mL (isolat M.II.4.1), 1,582 ± 0,199 µg/mL (isolat M.II.5.1), dan 5,129 ± 2,259 µg/mL (isolat M.II.6.1). Pada kondisi uji yang sama, klorokuin difosfat yang digunakan sebagai kontrol positif mempunyai IC_{50} sebesar 0,003 ± 0,0002 µg/mL. Ketiga isolat tersebut selanjutnya diidentifikasi secara kromatografi dan spektroskopi untuk mengetahui jenis senyawa atau golongan senyawa yang terkandung di dalamnya.

Pengujian aktivitas antimalaria dalam penelitian ini dilakukan secara *in-vitro* terhadap kultur non-sinkronisasi *P. falciparum* strain-3D7 dalam lempeng sumur-sumur mikro. Larutan ekstrak metanol, fraksi serta isolat-isolat hasil pemisahannya, masing-masing sebanyak 5 (lima) konsentrasi dalam pelarut DMSO, ditambahkan ke dalam kultur *Plasmodium*, dan diinkubasi selama 48 jam. Sebagai kontrol negatif digunakan DMSO, dan kontrol positif klorokuin difosfat. Setelah diinkubasi selama 48 jam, dibuat hapusan darah tipis di atas *slide* kaca, difiksasi dengan metanol dan diwarnai dengan Giemsa, untuk selanjutnya diamati dan dihitung jumlah eritrosit yang terinfeksi parasit di bawah mikroskop. Perhitungan jumlah eritrosit yang terinfeksi parasit ini dilakukan terhadap sekitar 5000-an eritrosit.

Data hasil pembacaan *slide* di bawah mikroskop adalah berupa perbandingan jumlah eritrosit yang terinfeksi parasit (*Plasmodium*) dengan jumlah total eritrosit yang dihitung. Hasil perhitungan ini dinyatakan sebagai persen parasitemia, yang kemudian diolah menjadi persen pertumbuhan dan persen penghambatan. Data persen

penghambatan selanjutnya diolah menggunakan analisis probit untuk menentukan aktivitas penghambatan masing-masing zat uji (ekstrak, fraksi, isolat) terhadap pertumbuhan *Plasmodium*, yang dinyatakan dengan nilai IC_{50}, yang besarnya masing-masing adalah seperti yang telah dipaparkan di atas.

Hasil identifikasi menggunakan metode KLT dengan penampak noda dan reaksi warna yang dilakukan terhadap isolat-isolat M.II.4.1, M.II.5.1 dan M.II.6.1 menunjukkan bahwa isolat-isolat tersebut mengandung senyawa fenolik. Hasil identifikasi dengan spektroskopi UV menunjukkan adanya ikatan rangkap terkonjugasi, yang yang merujuk pada ikatan-ikatan rangkap dalam diena atau poliena terkonjugasi, karbonil, karbonil α,β-tidak jenuh, atau cincin aromatik. Hasil identifikasi terhadap ketiga isolat dengan metode spektroskopi IR mengindikasikan adanya ikatan-ikatan O-H (hidroksil), C=C aromatik dan C=O (karbonil), sehingga disimpulkan ketiga isolat tersebut mengandung senyawa karbonil-fenolik. Analisis kemurnian isolat dengan menggunakan metode HPLC menunjukkan bahwa ketiga isolat tersebut masing-masing masih mengandung lebih dari satu senyawa (tidak murni).

Kesimpulan dari hasil penelitian ini adalah bahwa ekstrak metanol kulit batang *A. champeden* mengandung senyawa yang secara *in-vitro* aktif sebagai antimalaria (menghambat pertumbuhan *P. falciparum*). Untuk penelitian lanjutan, disarankan agar dapat dilakukan pemurnian terhadap isolat M.II.4.1 (isolat yang paling aktif) sehingga dapat ditentukan struktur senyawa yang aktif sebagai antimalaria tersebut.

SUMMARY

ISOLATION AND TEST OF ANTIMALARIAL ACTIVITY OF METHANOL EXTRACT, FRACTION AND ISOLATES OF CEMPEDAK (*Artocarpus champeden,* Spreng.) STEM BARK

Cempedak (*A. champeden*) stem bark has been used traditionally as an antimalarial medication. In order to be developed as an antimalarial medicine, as the first step, it is necessary to isolate the antimalarial active substance(s) contained in that part of plant.

The aim of this research was to obtain the antimalarial active substance(s) through the method of isolation guided by the antimalarial activity test at every level of separation of *A. champeden* stem bark extract. In this research, a series of extractions was done through the maceration of pulverized stem bark of *A. champeden* successively using hexane, dichloromethane, and methanol, for 1 x 3 x 24 hours respectively.

The methanol extract was then tested for its antimalarial activity against the non-synchronized 3D7-strain of *Plasmodium falciparum* using the *in-vitro* technique for 48 hours of incubation period. The IC_{50} value resulted from this test was 4.230 ± 1.147 µg/mL. The methanol extract was then fractionated with column chromatography. A pre-adsorbed of 12 grams of methanol extract was fractionated in a column containing slurries of 400 grams of silica gel using eluents of $CHCl_3$-EtOAc (1:9), $CHCl_3$-MeOH (9:1), $CHCl_3$-MeOH (8:2), $CHCl_3$-MeOH (1:1), and MeOH. Fractions obtained were then combined according to the similarity of their thin layer chromatography (TLC) profiles and by so doing 8 (eight) major fractions were produced. Each fraction was tested again for its antimalarial activity. The second fraction (M.II) demonstrated the highest degree of activity in inhibiting the growth of *Plasmodium,* with the IC_{50} value was 0.146 ± 0.046 µg/mL, and as such was chosen to be further separated or isolated.

The M.II fraction was then further separated using the preparative thin layer chromatography, and by so doing 8 (eight) subfractions were produced. From the subfractions of M.II.4, M.II.5 and M.II.6, one isolate of each of them in the form of amorphous solid was obtained, then named as M.II.4.1, M.II.5.1, and M.II.6.1. These isolates were then tested for their antimalarial activity, and found to have the IC_{50} values as follows : 0.052 ± 0.011 μg/mL (for M.II.4.1 isolate), 1.582 ± 0.199 μg/mL (for M.II.5.1 isolate), and 5.129 ± 2.259 μg/mL (for M.II.6.1 isolate). With the same test condition, the positive control chloroquine diphosphate has the IC_{50} of 0.003 ± 0.0002 μg/mL. These three isolate were then identified chromatographically and spectroscopically in order to know the type(s) of chemical compounds and chemical compound groups contained.

The test of antimalarial activity was done *in-vitro* with the non-synchronized 3D7-strain cultures of *P. falciparum* using microplates. The solution of methanol extract, fraction and isolates resulting from the separation process, each being 5 (five) concentrations in DMSO was added into *Plasmodium* cultures and incubated for 48 hours. DMSO was used as the negative control, and chloroquine diphosphate was used as the positive control. After the 48 hours of incubation period, a thinly smear were prepared from each well upon a glass slide, fixed with methanol and stained with Giemsa prior to observation under the ligt microscope. The number of infested-erythrocytes was counted per 5,000s erythrocytes.

The percentage of parasitemia as the percentage of parasite growth was calculated based on the number of infected-erythrocytes per 5,000s erythrocytes. Then, this data was used to calculate the percentage of inhibition of parasite growth, and then computerized for probit analysys to obtain IC_{50} value.

The result of the identification of M.II.4.1, M.II.5.1 and M.II.6.1 isolates employing the method of thin layer chromatography showed that the isolates contained a phenolic compound. Identification with the UV spectroscopic showed some signal of conjugated unsaturation bond(s), those refered to unsaturation bonds

in conjugated dienes or polyenes, carbonyl groups, α,β-unsaturated carbonyl groups, or aromatic rings. Identification with IR spectrophotometer found that there were O-H, aromatic C=C and C=O bonds in each isolate. So, therefore, each isolate contained a phenolic-carbonyl compound. Analysis of the purity of those isolates using HPLC method showed that the isolates still contained more than one compound (not pure).

The conclusion of this research was that the methanol extract of *A. champeden* stem bark contained one or more *in-vitro* active antimalarial compound(s). For further research, it is suggested that advanced separation and purification can be carried out for the the most active M.II.4.1 isolate until a pure compound is obtained, so that the structure of it's antimalarial compound can be determined.

ABSTRACT

ISOLATION AND TEST OF ANTIMALARIAL ACTIVITY OF METHANOL EXTRACT, FRACTION AND ISOLATES OF CEMPEDAK (*Artocarpus champeden,* Spreng.) STEM BARK

This research was conducted to obtain the antimalarial active substance(s) from methanol extract of *A. champeden* stem bark through the method of isolation guided by the antimalarial activity test at every level of methanol extract separations. The separation or isolation of methanol extract was conducted through the method of column chromathography and preparative thin layer chromatography. The tests of antimalarial activity of methanol extract, fractions and isolates were done *in-vitro* with the non-synchronized culture of 3D7-strain of *P. falciparum* for 48 hours period of incubation, with DMSO as a negative control and chloroquine diphosphate as a positive control. First, methanol extract was tested for its antimalarial activity, based on the observation on Giemsa-stained thin smears, and it was found the IC_{50} value of 4.230 ± 1.147 µg/mL. This extract was then fractionated, and fractions produced were tested once again for their antimalarial activity. Fraction indicating the highest degree of antimalarial activity was the M.II fraction, with the IC_{50} value of 0.146 ± 0.046 µg/mL, was then further separated to obtain isolates. Three amorphous isolates were obtained from this M.II fraction, named as M.II.4.1, M.II.5.1 and M.II.6.1 isolates. These isolates were also tested for their antimalarial activity, and found to have the IC_{50} values of 0.052 ± 0.011 µg/mL, 1.582 ± 0.199 µg/mL, and 5.129 ± 2.259 µg/mL, respectively. With the same test condition, chloroquine diphosphate has the IC_{50} value of 0.003 ± 0.0002 µg/mL. The result of identification of the three isolates demonstrated the existence of phenolic-carbonyl compound contained in each isolate. The conclusion of this research was that the methanol extract of *A. champeden* stem bark contained the compound that was *in-vitro* active as antimalarial.

Key words : *A. champeden* stem bark, methanol extract,fraction and isolates, *in-vitro* antimalarial test, *P.falciparum* strain-3D7.