

Karakterisasi enzim malat:kinon oksidoreduktase dari plasmodium falciparum (PfMQO); EC: 1.1.5.4 dan penghambatan aktivitasnya oleh  $\alpha$ -mangostin sebagai kandidat antimalaria = Characterization of malate quinone oxidoreductase enzyme from plasmodium falciparum (PfMQO); EC 1.1. 5. 4 and activity inhibition by mangostin as an antimalaria candidate

Endah Dwi Hartuti, author

Deskripsi Lengkap: <https://lib.ui.ac.id/detail?id=20455861&lokasi=lokal>

---

Abstrak

Latar belakang: Plasmodium falciparum merupakan salah satu parasit penyebab penyakit malaria yang menyerang manusia. Mitokondria P. falciparum memiliki perbedaan komponen dengan manusia terutama jenis enzim yang terlibat dalam rantai transpor elektron. Malat: kinon oksidoreduktase MQO adalah enzim fungsional yang bekerja dalam mengkatalisis konversi malat menjadi oksaloasetat yang dibutuhkan pada siklus asam sitrat. Elektron yang dihasilkan akan dimanfaatkan untuk pembentukan ATP melalui fosforilasi oksidatif.  $\alpha$ -mangostin merupakan senyawa xanton utama yang berasal dari manggis, Garcinia mangostana Linn. Ekstrak kulit buah manggis dan  $\alpha$ -mangostin diketahui memiliki efek antiplasmodium yang dibuktikan melalui penelitian in vitro.

Metode: Enzim rekombinan PfMQO diekspresikan pada bakteri Escherichia coli BL21star DE3 . Fraksi membran E.coli yang mengikat enzim diisolasi menggunakan sentrifugasi kecepatan 104.000xg.

Karakteristik enzim PfMQO ditentukan secara spektrofotometri dengan mengikuti kinetika reaksi enzim terhadap substrat malat dan ubikinon pada 600 nm. Aktivitas penghambatan  $\alpha$ -mangostin terhadap PfMQO diuji dengan mengikuti reduksi ubikinon pada panjang gelombang 278 nm. Uji konfirmasi aktivitas inhibisi  $\alpha$ -mangostin dilakukan terhadap kultur P. falciparum in vitro dan sel limfosit manusia.

Hasil: PfMQO bekerja pada kondisi optimal di suhu 37 C dan pH netral. Enzim PfMQO yang terikat pada fraksi membran bakteri memiliki karakter nilai aktivitas spesifik sebesar 13,3890  $\mu$ mol/menit/mg, konstanta Michaelis-Menten Km untuk ubikinon sebesar 6,2090 0,6486  $\mu$ M dan Vmax sebesar 16,9600 0,5866  $\mu$ mol/menit/mg . Nilai konstanta Michaelis-Menten Km untuk malat sebesar 5,9960 0,3440 mM dan Vmax sebesar 16,4000 0,3838  $\mu$ mol/menit/mg .  $\alpha$ -mangostin memiliki aktivitas penghambatan terhadap enzim PfMQO dengan nilai IC50 sebesar 1,7390 0,0077 M. Penghambatan  $\alpha$ -mangostin terhadap enzim PfMQO di situs pengikatan malat dan ubikinon melalui mekanisme campuran dengan nilai konstanta inhibisi Ki masing-masing sebesar 2,3260 mM dan 1,6720  $\mu$ M.  $\alpha$ -mangostin memiliki aktivitas penghambatan pertumbuhan terhadap kultur P. falciparum IC50 = 5,7060 1,0976 M . Uji toksisitas  $\alpha$ -mangostin terhadap sel limfosit manusia memberikan nilai hambatan CC50 sebesar 11,3800  $\mu$ M. Evaluasi toksisitas  $\alpha$ -mangostin melalui nilai perhitungan SI didapatkan SI terhadap PfMQO sebesar 6,5440 dan kultur P. falciparum 1,9944. Kesimpulan: Enzim MQO dalam tubuh parasit P. falciparum dapat ditetapkan sebagai target pengobatan malaria dan  $\alpha$ -mangostin berpotensi untuk dikembangkan sebagai antimalaria namun masih bersifat toksik bagi sel manusia.

Background Plasmodium falciparum is one of parasite causing malaria disease that attacks human. Mitochondria of P. falciparum has a different component with human especially enzyme that involve in electron transport chain. Malate quinone oxidoreductase MQO is functional enzyme which catalyze

conversion of malate to oxaloacetate which needed in citric acid cycle. Generated electron will be utilized to form ATP through oxidative phosphorylation. a mangostin is a main xanton of mangosteen, *Garcinia mangostana* Linn. Mangosteen pericarp extract and a mangostin have been known in having antiplasmodial effect by in vitro study.

Method PfMQO recombinant enzyme was expressed in bacteria *Escherichia coli* BL21star DE3 . *E.coli* membrane fraction that expressed enzyme were isolated using centrifugation 104.000 x g. Characterization of PfMQO were determined by spectrophotometry with following kinetic reaction of enzyme to malate and ubiquinone as substrate at 600 nm. Inhibition activity mangostin against PfMQO were assayed by following ubiquinone reduction at 278 nm. Confirmation test of mangostin inhibition activity were conducted againts *Plasmodium falciparum* culture in vitro and human lymphocyte cell.

Results PfMQO has an optimum condition at 37 C and netural pH. PfMQO enzyme which bind on bacterial membrane fraction has a specific activity 13.3890 mol minutes mg, Michaelis Menten value Km for ubiquinone is 6.2090 0.6486 M and Vmax is 16.9600 0.5866 mol minutes mg . Michaelis Menten value Km for malate is 5.9960 0.3440 mM and Vmax is 16.4000 0.3838 mol minutes mg . mangostin has inhibition activity against PfMQO enzyme with inhibition concentration IC50 value of 1.7390 0.0077 M. Inhibition of mangostin to PfMQO at malate and ubiquinone binding site by mixed type inhibition with inhibition constant Ki values are 2.3260 mM and 1.6720 M, respectively. mangostin has inhibition activity to *P. falciparum* growth with IC50 value of 5.7060 1.0976 M. Toxicity value CC50 to human lymphocyte cells is 11.3800 M. Evaluation of mangostin toxicity based on SI with SI value to PfMQO of 6,5440 and to *P. falciparum* culture of 1,9944.

Conclusion PfMQO enzyme in parasite *P. falciparum* body could be determined as malaria drug target and a mangostin is potential to be developed as antimalarial agent but still toxic for human cell.