Penentuan Aktivitas Inhibitor Enzim α-Glukosidase Ekstrak Etil Asetat Bakteri Endofit yang Diisolasi dari Daun Tapak dara (*Catharanthus roseus* (L.) G. Don) secara In Vitro

TESIS

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Abstrak

Daun tapak dara (*Catharanthus roseus* (L.) G. Don) dikenal dapat menghasilkan senyawa bioktif yang bermanfaat secara terapetik, salah satunya sebagai agen inhibitor terhadap enzim α-glukosidase pada penyakit diabetes. Interaksi antara mikroorganisme endofit dan tamanan inangnya telah dipelajari secara luas karena dapat menghasilkan berbagai macam metabolit sekunder yang memiliki fungsi biologis signifikan dari inangnya. Penelitian ini bertujuan untuk mengetahui potensi senyawa hasil isolasi dari ekstrak etil asetat bakteri endofit daun tapak dara dalam menghambat enzim α-glukosidase. Bakteri endofit ditumbuhkan dan diisolasi dalam media *Nutrient agar.* Bakteri yang telah murni difermentasi selama 24-72 jam pada media *nutrient broth*. Etil asetat digunakan untuk mengekstrak metabolit hasil fermentasi. Skrining bioaktivitas inhibitor enzim α-glukosidase dari ekstrak diuji dengan metode KLT bioautografi dan penentuan nilai IC_{50}. Pendekaan profil kandungan kimia dari ekstrak dilakukan dengan metode LC-MS-MS. Senyawa diisolasi dari ekstrak aktif dengan menggunakan kolom Sephadex LH-20. Hasil evaluasi menunjukkan bahwa ekstrak etil asetat memberikan nilai IC_{50} yang kuat terhadap 3 isolat dari 8 isolat bakteri endofit yaitu (TD 1, TD3, TD 4) masing masing sebesar 19,937 µg/ml, 92,3428 µg/ml, dan 13,4910 µg/ml dan pembanding acarbose sebesar 48,859 µg/ml. Terdapat 4 senyawa yang teridentifikasi dari ekstrak etil asetat bakteri endofit (TD1) dengan membandingkan data massa molekul dari MS/MS dengan literatur. Isolat bakteri diidentifikasi secara molekuler sebagai *Pseudomonas oryzihabitans* (Isolat TD 1) dan *Staphylococcus warneri* (Isolat TD 4) pada bakteri yang potensial. Senyawa hasil isolasi yang diperoleh dari karakterisasi dengan LC-MS/MS sebagai (Cyclopeptides) cyclo-(L-Phe-L-Pro). Senyawa aktif ini menghambat enzim alfa glukosidase dengan nilai IC_{50} 26,762 µg/ml. Berdasarkan hasil penelitian, dapat disimpulkan bahwa bakteri endofit dari daun tapak dara berpotensi menghasilkan senyawa sebagai inhibitor enzim α-glukosidase.

Kata Kunci: α-glukosidase, bakteri endofit, *Catharanthus roseus* (L.) G. Don, inhibitor enzim, IC_{50}, LC-MS/MS

The Determination of α-Glucosidase Enzyme Inhibitory Activity of Ethyl Acetate Extract from Endophytic Bacteria Isolated from the Leaves of Madagascar Periwinkle (*Catharanthus roseus* (L.) G. Don) In Vitro

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Abstract

The leaves of Madagascar periwinkle (Catharanthus roseus (L.) G. Don) are known to produce bioactive compounds with therapeutic benefits, one of which is their role as inhibitors of the enzyme α-glucosidase, useful in diabetes treatment. The interaction between endophytic microorganisms and their host plants has been widely studied because it can lead to the production of various secondary metabolites with significant biological functions derived from the host. This study aims to investigate the potential of isolated compounds from ethyl acetate extracts of endophytic bacteria from Madagascar periwinkle leaves in inhibiting the α-glucosidase enzyme. The endophytic bacteria were cultured and isolated on nutrient agar medium. Pure bacterial isolates were fermented for 24–72 hours in nutrient broth medium. Ethyl acetate was used to extract the metabolites from the fermentation. Bioactivity screening for α-glucosidase inhibitory activity from the extracts was conducted using the Thin Layer Chromatography (TLC) bioautography method and IC50 value determination. Chemical profiling of the extracts was performed using LC-MS-MS. Compounds were isolated from active extracts using a Sephadex LH-20 column. The evaluation results showed that the ethyl acetate extract produced a strong IC50 value against 3 of the 8 isolates of endophytic bacteria, namely (TD 1, TD 3, TD 4), with values of 19.937 µg/ml, 92.3428 µg/ml, and 13.4910 µg/ml, respectively, in comparison to acarbose at 48.859 µg/ml. Four compounds were identified from the ethyl acetate extract of endophytic bacteria (TD 1) by comparing the molecular mass data from MS/MS with literature. The bacterial isolates were molecularly identified as Pseudomonas oryzihabitans (Isolate TD 1) and Staphylococcus warneri (Isolate TD 4) among the potential bacteria. The isolated compound characterized by LC-MS/MS as cyclopeptides, specifically cyclo-(L-Phe-L-Pro), showed an IC50 value of 26.762 µg/ml for α-glucosidase inhibition. Based on the results, it can be concluded that endophytic bacteria from Madagascar periwinkle leaves have the potential to produce compounds as α-glucosidase enzyme inhibitors.

Keyword: *α-glucosidase enzyme, endophytic bacteria, Catharanthus roseus* (L.) G. Don, *inhibitor, IC50, LC-MS/MS*