

Identifikasi Senyawa Bioaktif yang Memiliki Aktivitas Anti-elastase dan Antioksidan dari Ekstrak Metanol Kulit Batang Tanaman Mabolo (*Diospyros blancoi* A. DC.) = Identification of Bioactive Compounds with Anti-elastase and Antioxidant Activities from Methanol Extract of Mabolo Plant Stem Bark (*Diospyros blancoi* A. DC.)

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Abstrak

Riset pencarian senyawa bioaktif yang memiliki aktivitas penghambatan terhadap enzim elastase dan antioksidan dalam menunda proses penuaan pada kulit terus diupayakan agar dapat dikembangkan menjadi kandidat agen anti-aging yang potensial. Tanaman mabolo (*Diospyros blancoi* A. DC.) merupakan tanaman asli Indonesia yang belum banyak dieksplorasi pemanfaatannya baik secara tradisional maupun ilmiah sebagai anti-aging. Penelitian ini bertujuan untuk mengidentifikasi senyawa bioaktif dari ekstrak metanol daun (EDM) atau ekstrak metanol kulit batang (EKM) tanaman mabolo yang memiliki aktivitas anti-elastase dan antioksidan teraktif. Ekstraksi daun dan kulit batang dengan pelarut metanol menggunakan ultrasound assisted-extraction (UAE). Aktivitas anti-elastase dan antioksidan dari tiap ekstrak, fraksi, subfraksi, dan isolat dievaluasi. Hasil penelitian menunjukkan bahwa EKM memiliki aktivitas anti-elastase dan antioksidan lebih kuat dibanding EDM meskipun keduanya memiliki aktivitas yang sangat kuat dibawah 50 µg/mL. EKM sebagai ekstrak teraktif dilanjutkan fraksinasi padat-cair dan menghasilkan fraksi etil asetat kulit batang mabolo (FEKM) yang memiliki aktivitas anti-elastase dan antioksidan paling kuat dibandingkan dengan fraksi metanol kulit batang mabolo (FMKM) maupun fraksi heksan kulit batang mabolo (FHKM). FEKM sebagai fraksi teraktif difraksinasi lebih lanjut dengan kromatografi kolom menghasilkan 16 subfraksi etil asetat kulit batang mabolo (SEKM A-SEKM P). Hasil identifikasi isolat melalui FTIR dan NMR dibandingkan terhadap literatur. Isolat DB1 yang diperoleh dari isolasi SEKM N diduga asam galat, sedangkan isolat DB2 yang diperoleh dari isolasi SEKM L dan SEKM N diduga asam betulinat. Aktivitas anti-elastase isolat DB2 [IC₅₀ = 8,62 µg/mL (18,88 µM)] lebih kuat dibanding DB1 (IC₅₀ = 93,35 µg/mL (548,70 µM)]. Akan tetapi, aktivitas antioksidan isolat DB1 lebih kuat dibanding isolat DB2. Sementara itu, aktivitas antielastase isolat DB2 menunjukkan jenis inhibisi unkompetitif secara in vitro. Hasil interaksi secara in silico diprediksi isolat DB2 menghambat bukan pada sisi aktif enzim elastase (1B0F) dengan energi bebas ikatan sebesar -5,85 kcal/mol.

.....Studies on the discovery of bioactive compounds that have inhibitory activity against elastase enzymes and antioxidants in delaying the skin's aging process are still being explored so that potential anti-aging agents could be developed. The mabolo plant (*Diospyros blancoi* A. DC.) is a native Indonesian plant that has not been widely explored for its traditional and scientific uses as anti-aging. This study aimed to identify bioactive compounds from leaf methanol extract (EDM) or stem bark methanol extract (EKM) of the mabolo plant that have the most active anti-elastase and antioxidant activities. Extraction of leaves and stem bark with methanol solvent using ultrasound assisted-extraction (UAE). Both the anti-elastase and antioxidant activities of each extract, fraction, subfraction, and isolate were evaluated. The results showed that EKM had stronger anti-elastase and antioxidant activities than EDM although both had very strong activities below 50 µg/mL. EKM as the most active extract was followed by solid-liquid fractionation and

resulted in ethyl acetate fraction of mabolo stem bark (FEKM) which had the strongest anti-elastase and antioxidant activities compared to methanol fraction of mabolo stem bark (FMKM) and hexane fraction of mabolo stem bark (FHKM). FEKM as the most active fraction was further fractionated by column chromatography to obtain 16 ethyl acetate subfractions of mabolo stem bark (SEKM A - SEKM P). The results of isolate identification by FTIR and NMR were compared to the literature. Isolate DB1 obtained from SEKM N isolation was considered gallic acid, while isolate DB2 obtained from SEKM L and SEKM N isolation was considered betulinic acid. The anti-elastase activity of isolate DB2 [IC₅₀ = 8.62 µg/mL (18.88 µM)] was stronger than DB1 (IC₅₀ = 93.35 µg/mL (548.70 µM)). However, the antioxidant activity of isolate DB1 was stronger than isolate DB2. Meanwhile, the anti-elastase activity of isolate DB2 showed an uncompetitive type of inhibition *in vitro*. *In silico* interaction results predicted DB2 isolate inhibits not on the active side of the elastase enzyme (1B0F) with a binding free energy of -5.85 kcal/mol.