

Profil metabolit sekunder ekstrak etanol 70 daun bandotan *Ageratum conyzoides* L. dan potensinya dalam menghambat transkripsi gen inflamasi pada sel RAW 264.7 terinduksi lipopolisakarida = Secondary metabolites profiles of 70 ethanol extract of bandotan leaves *Ageratum conyzoides* L and its potency to inhibits the transcription of inflammatory gene in RAW 264 7 stimulated by lipopolysaccharides

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Abstrak

Bandotan *Ageratum conyzoides* L. merupakan salah satu tanaman herbal Indonesia yang banyak digunakan dalam pengobatan tradisional, salah satunya dalam terapi peradangan inflamasi. Penelitian sebelumnya mengungkapkan bahwa isolat kuersetin dari ekstrak daun bandotan memiliki aktivitas anti-inflamasi.

Namun, dibutuhkan waktu yang lama dalam proses ekstraksi.

Penelitian bertujuan mencari metode ekstraksi yang cocok yang dapat mempersingkat waktu ekstraksi dan meningkatkan kadar kuersetin dalam ekstrak, serta bertujuan menginvestigasi mekanisme molekuler anti-inflamasi dari ekstrak. Kuersetin, methotrexate dan piroxicam digunakan sebagai kontrol positif.

Metode ekstraksi yang digunakan adalah maserasi dan digesti, dengan air dan etanol 70 sebagai pelarut.

Profil metabolit sekunder dianalisis dengan kromatografi lapis tipis KLT dan Liquid Chromatography-Mass Spectroscopy LC-MS. Aktivitas anti inflamasi dari ekstrak dievaluasi dengan sel RAW 264.7 distimulasi oleh lipopolisakarida LPS dan dilakukan deteksi ekspresi gen-gen dengan Reverse Transcription-Polymerase Chain Reaction RT-PCR ditingkat messenger ribonucleic acid mRNA. Uji aktivitas juga dilakukan terhadap nitrit oksida NO dengan metode Griess.

Hasil uji memperlihatkan bahwa kadar kuersetin tertinggi 52,71 ppm diperoleh dari metode digesti pada suhu 60 C selama 2 jam dengan pelarut etanol 70 . Kromatogram KLT menunjukkan pola yang khas dan kromatogram LC-MS memperlihatkan beberapa puncak metabolit sekunder, salah satunya adalah kuersetin.

Pada dosis 50 µg/ mL, ekstrak dapat menurunkan ekspresi messenger ribonucleic acid mRNA cyclooxygenase-2 COX-2 , tumor necrosis factor-α TNF-α , interleukin-1β IL-1β , IL-6, dan nuclear factor-κβ NF-κβ , serta menurunkan produksi NO. Berdasarkan hasil yang diperoleh, disimpulkan bahwa ekstrak etanol 70 daun bandotan memiliki mekanisme aksi anti-inflamasi seperti kuersetin dalam menekan mediator pro-inflamasi.

.....Bandotan *Ageratum conyzoides* L. is one of Indonesian herbs are widely used in traditional medicines one of them is in treating inflammation. Previous research has revealed that the isolated quercetin from bandotan leaves extract has anti inflammatory activity. However, the extraction process takes a long time. The aim of the present study was to find the suitable method which can reduce the time of extraction process and also increase quercetin content in extract, and also investigates the anti inflammatory molecular mechanism of extract. Quercetin, methotrexate, and piroxicam were used as positive control. Two extraction methods were used maceration and digestion method, which used water and ethanol 70 as a solvent. Secondary metabolites profiles were analyzed by thin layer chromatography TLC and liquid chromatography mass spectroscopy LC MS . The anti inflammatory activity of extract was evaluated using RAW 264.7 cells stimulated by lipopolysaccharides LPS and the genes were detected by reverse

transcription polymerase chain reaction RT PCR at messenger ribonucleic acid mRNA . The activity test was also performed on nitric oxide NO by Griess method.

The results showed that the highest quercetin content 52.71 ppm was obtained from digestion method at 60 C for 2 hours with ethanol 70 as a solvent. TLC chromatograms shows a typical pattern and LC MS chromatograms shows some peaks of secondary metabolites, one of them is quercetin. The dose extract at 50 g mL can decrease mRNA expression of cyclooxygenase 2 COX 2 , tumor necrosis factor TNF , interleukin 1beta IL 1 , IL 6, dan nuclear factor kappa betha NF , and also can decrease of NO production. As a result, it is concluded that 70 ethanolic leaves extract of bandotan has anti inflammatory activity such as quercetin in suppressing pro inflammatory mediators.