

INTISARI

IDENTIFIKASI dan KARAKTERISASI ANTIOKSIDAN dari JUS *ALOE CHINENSIS* dan EVALUASI POTENSI ALOE-EMODIN sebagai ANTIFOTOOKSIDAN dalam SISTEM ASAM LINOLEAT

Telah dilakukan identifikasi dan karakterisasi antioksidan dari jus *Aloe chinensis* dan evaluasi potensi aloe-emodin sebagai antifotooksidan dalam sistem asam linoleat. Jus *Aloe chinensis* dibedakan menjadi 2 jus keruh dan jus jernih kemudian dievaluasi kemampuannya sebagai penangkap radikal dalam sistem asam linoleat, liposom dan DPPH serta dihitung kandungan total fenolnya. Jus keruh *Aloe chinensis* dievaluasi stabilitas antioksidannya (aktivitas antiradikal, antiperoksidasi asam linoleat, antifotooksidasi dan kandungan total fenol) terhadap perlakuan panas (kontrol, 60, 80, dan 100 °C selama 30 menit). Jus keruh *Aloe chinensis* kering beku diekstrak dengan pelarut metanol, etanol dan aseton. Komponen antioksidan dalam ekstrak metanol *Aloe chinensis* dipisahkan dengan kolom kromatografi silika gel G-60 dan fase gerak etil asetat/metanol secara gradien elusi. Fraksi II menghasilkan aktivitas antiradikal dan antifotooksidasi yang tinggi kemudian dimurnikan dengan kolom yang sama dan menghasilkan subfraksi II-b, subfraksi II-c dan subfraksi II-d. Ketiga subfraksi dipisahkan dalam plat kromatografi lapis tipis silika gel F₂₅₄ dan subfraksi II-b menghasilkan noda kuning tunggal dan menunjukkan R_f yang sama dengan standar aloe emodin. Penentuan struktur antioksidan dilakukan berdasarkan serapan spektra UV, massa dan pola kromatogram dari kromatografi cair. Dilakukan pengujian terhadap pengaruh konsentrasi metilen biru, aloe emodin, asam linoleat terhadap pembentukan peroksida selama fotooksidasi. Kinetika dan mekanisme penstabilan oksigen singlet oleh aloe emodin dipelajari dengan menggunakan metode kinetika *steady state*.

Jus *Aloe chinensis* menunjukkan aktivitas sebagai penangkap radikal dalam sistem asam linoleat, liposom dan DPPH dan aktivitasnya dipengaruhi oleh cara preparasi dan kandungan jus dalam sistem. Jus keruh *Aloe chinensis* menunjukkan aktivitas yang lebih tinggi dibanding jus jernih pada konsentrasi yang sama. Peningkatan konsentrasi karbon aktif untuk penjernihan jus *Aloe chinensis* mengakibatkan penurunan aktivitas antiradikal. Aktivitas antiradikal jus *Aloe chinensis* tidak dipengaruhi oleh kandungan total fenolnya. Pemanasan jus *Aloe chinensis* menghasilkan penurunan aktivitas antiradikal, antiperoksidasi asam linoleat, antifotooksidasi dan kandungan fenol. Pemanasan pada 100 °C menunjukkan aktivitas antiopksidasi dan kandungan total fenol paling rendah. Komponen antioksidan dalam ekstrak metanol *Aloe chinensis* dipisahkan dengan kolom kromatografi silika gel G 60 dengan eluen etil asetat/metanol. Fraksi II menghasilkan aktivitas antiradikal dan antifotooksidasi tinggi dan dimurnikan sehingga diperoleh 3 subfraksi II-b, II-c dan subfraksi II-d. Subfraksi II-b menghasilkan spot kuning tunggal pada pemisahan noda dengan KLT dan menunjukkan R_f yang sama dengan standar aloe emodin. Spektra massa subfraksi II-b menunjukkan berat molekul 256 dan pola kromatogram yang sama dengan standar aloe-emodin pada kromatografi cair. Struktur antioksidan dalam *Aloe chinensis* diidentifikasi sebagai 1,8 dihidroksi-3-hidroksimetil antron



emodin antrone). Peningkatan konsentrasi metilen blue dan asam linoleat meningkatkan pembentukan peroksida selama fotooksidasi dan sebaliknya pada peningkatan konsentrasi aloe-emodin.

Jus *Aloe chinensis* berkemampuan sebagai antioksidan melalui mekanisme terminasi radikal bebas. Kemampuan aktivitasnya dipengaruhi oleh cara preparasi jus dan kandungan jus dalam sistem. Stabilitas antioksidan dalam jus *Aloe chinensis* dipengaruhi oleh suhu pemanasan. Pemanasan mengakibatkan penurunan aktivitas antioksidasi dan kandungan total fenol. Senyawa antioksidan yang teridentifikasi dalam ekstrak metanol *Aloe chinensis* adalah 1,8 dihidroksi-3-hidroksimetil antron (aloe emodin antrone). Aloe emodin berkemampuan sebagai antifotooksidan dalam sistem asam linoleat dan aktivitasnya dipengaruhi oleh konsentrasi metilen blue, aloe emodin dan asam linoleat. Kecepatan penstabilan total oksigen singlet oleh aloe-emodin dalam sistem asam linoleat adalah $4.68 \times 10^3 \text{ M}^{-1} \text{detik}^{-1}$. Aloe emodin berkemampuan mereduksi fotooksidasi asam linoleat melalui mekanisme penstabilan oksigen singlet dan penstabilan sensitiser triplet tereksitasi.

ABSTRACT

IDENTIFICATION and CHARACTERIZATION of ANTIOXIDANT from *ALOE CHINENSIS* and EVALUATION of ALOE EMODIN as ANTIPHOTOOXIDANT in LINOLEIC ACID SYSTEMS

Identification and characterization of antioxidant of phenolics compound from *Aloe chinensis* and evaluation of Aloe emodin as anti-photooxidant was investigated. *Aloe chinensis* juice divided into two treatment cloudy and clarified juice. A scavenging radical activity assay was carried out by using the linoleic acid and liposome and in DPPH systems. The total of phenol contents in the juice was measured by the method of Folin Ciocalteu. Effect of thermal treatment of *Aloe chinensis* cloudy juices (raw, cooked at 60, 80 and 100 °C for 30 minutes) was to investigate antiradical, antiperoxide of linoleic acid, anti-photooxidant activity and the total of phenolics. Antioxidant compounds was extracted from *Aloe chinensis* juice using methanol, ethanol and acetone. Each extract was measuring antiradical and anti-photooxidant activity. The methanolic extracts was fractionated onto chromatographed silica gel G-60 column. Ethyl acetate/methanol was used as gradient eluent solvent. The fraction II that exhibited highest antiradical and anti-photooxidant activity was loaded on silica gel G-60 column. Sub fractions II-b, II-c and II-d that exhibited antiradical and anti-photooxidant activity were further investigated. Sub fraction was purification by Thin-layer chromatography (TLC) methodology on silica gel GF₂₅₄ plates. The sub fraction II-b that showed a yellow single spots was investigated by mass spectrometry and absorption band in UV spectrum. The tentative identification of this compound was made by comparison with the HPLC. To study the effects of methylene blue, aloe emodin and linoleic acid concentration on the photosensitized oxidation were prepared in methylene chloride systems. The oxidation of linoleic acid was determined by measuring the peroxide value. The quenching mechanism and kinetics of aloe emodin in methylene blue-sensitized photooxidation of linoleic acid were studied by the steady-state kinetic methods.

Aloe chinensis juices was exhibited antioxidant activity in linoleic acid, liposome and DPPH systems, the activity was affected by the content of freeze dried juice in the system. At the same content of the freeze dried juice in the systems, *Aloe chinensis* cloudy juice showed higher activity than clarified juice on inhibition of linoleic acid peroxidation, scavenging DPPH radical activity and inhibition of malondyaldehyde formation. Increasing of the activated carbon that used for clarification of juices was produced decreasing antiradical activity of *Aloe chinensis* juices. Antiradical activity of *Aloe chinensis* cloudy juices was not affected by the total phenolic contents. Heating treatment of *Aloe chinensis* cloudy juice was produced decreasing antiradical, antiperoxide of linoleic acid, anti-photooxidant activity and the total of phenolic contents. Heat treatment of *Aloe chinensis* cloudy juice at 100 °C was exhibited lower on all assays than others. A potent antiradical and anti-photooxidant compound was separated on Silica gel column by the methanolic extract from *Aloe chinensis*. Fraction II possessed significant antiradical and anti-photooxidant activity and it was purified by the same columns. The sub fraction II-b exhibited stronger antiradical and



antiphotooxidant activity than others and showed a single spot in TLC. On the basis of mass spectrophotometry (m/z 256) in combination with reversed phased, high performance chromatography, this compound has been identified as 1,8 dihydroxy-3-hydroxymethyl anthrone (aloe emodin anthrone). The methylene blue and linoleic acid concentration were produced significantly increased of peroxide value on singlet oxygen oxydation of linoleic acid. The effect of aloe emodin concentration on singlet oxygen oxidation of linoleic acid was produced significantly decreased of peroxide value of linoleic acid. The calculated quenching rate of aloe emodin was $4.68 \times 10^9 \text{ M}^{-1}\text{S}^{-1}$. Aloe emodin both of quenched singlet oxygen and sensitizer to reduce photosensitized oxidation of linoleic acid by the singlet oxygen quenching mechanism and the excited triplet oxygen sensitizer quenching mechanism. The ability of antiphotooxidant activity of aloe emodin was affected by concentration.

Base of these results, termination of free radical reactions in cloudy and clarified of *Aloe chinensis* juices is responsible for the antioxidant activity. Heating treatment was affected stabilization of antioxidant action of *Aloe chinensis* cloudy juice. A potent antioxidant compound in *Aloe chinensis* has been identified as 1,8 dihydroxy-3-hydroxymethyl anthrone (aloe emodin anthrone). Aloe emodin was both of quenched singlet oxygen and sensitizer to reduce photosensitized oxidation of linoleic acid by the singlet oxygen quenching mechanism and the excited triplet oxygen sensitizer quenching mechanism. The ability of antiphotooxidant activity of aloe emodin was affected by concentration. The calculated quenching rate of aloe emodin was $4.68 \times 10^9 \text{ M}^{-1}\text{S}^{-1}$.