

Aplikasi Metode Ekstraksi Microwave Dengan Cairan Ionik Untuk Detoksifikasi Alkaloid Pirolizidin Daun Komfrey (*Symphytum officinale* L.) Dan Uji Hepatotoksitas Secara In-vitro = Application of Ionic Liquids-Microwave Assisted Extraction (IL-MAE) Method For Detoxification of Comfrey Leaves of Pyrrolizidine Alkaloid (*Symphytum officinale* L.) and In-vitro Hepatotoxicity test

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Abstrak

Tanaman komfrey (*Symphytum officinale* L.) mengandung allantoin, senyawa fenol asam rosmarinat, alkaloid pirolizidin dan derivatnya, yang telah digunakan pada pengobatan patah tulang, kerusakan tendon, ulserasi di saluran pencernaan dan edema paru. Aplikasi metode ekstraksi cairan ionik dengan microwave dikembangkan untuk mendapatkan kandungan bioaktif tersebut dari daun komfrey. Tujuan penelitian ini adalah mendapatkan parameter kondisi ekstraksi daun komfrey yang optimum untuk menarik alkaloid pirolizidin yang diwakili oleh retrorsin JNT-oksida dan senyawa allantoin, serta mengevaluasi toksisitasnya secara in vitro dengan sel HepaRg dari ekstrak daun komfrey yang optimum. Daun komfrey diekstraksi dengan 8 cairan ionik secara microwave assisted extraction (MAE) dan dilakukan skrining terhadap kadar fenol total, retrorsin iST-oksida dan allantoin. Cairan ionik terbaik dari hasil skrining dilakukan optimasi respon surface methodology (RSM) dengan desain Box-Behnken terhadap empat faktor dan tiga level yang digunakan untuk menentukan kondisi ekstraksi yang optimum terhadap kadar retrorsin iV-oksida dan allantoin. Ekstrak cairan ionik hasil optimasi terbaik dilakukan evaluasi terhadap aktivitas antioksidan DPPH dan ABTS, serta uji toksisitas in-vitro dengan sel HepaRg. Hasil dari skrining cairan ionik, diperoleh [BMIM]Br merupakan cairan ionik terbaik dengan kadar fenol total $0,01672 \pm 0,001$ mg/g serbuk, retrorsin α -oksida $0,049 \pm 0,007$ mg/g serbuk dan allantoin $1,335 \pm 0,243$ mg/g serbuk. Hasil optimasi ekstraksi dari cairan ionik [BMIM]Br diperoleh kadar retrorsin Noksida tertinggi pada run ke-24, yaitu $40,653 \pm 0,002$ pg/g serbuk dan kadar allantoin tertinggi pada run ke 27 yaitu $1,282 \pm 0,0137$ mg/g serbuk. Hasil optimasi ekstraksi dari cairan ionik [BMIM]Br memberikan kondisi optimum pada konsentrasi pelarut [BMIM]Br 1,16 mol/L, rasio pelarut/serbuk 20,98 mL/g, power 45,09%, dan waktu ekstraksi 12,07 menit, diperoleh kadar retrorsin JV-oksida 31,73 pg/g serbuk dan allantoin 0,809 mg/g. Aktivitas antioksidan baik DPPH dan ABTS menunjukkan nilai terendah pada ekstrak [BMIM]Br dengan IC₅₀ masing-masing 555,102 pg/mL dan 706,232 pg/mL. Nilai LC₅₀ untuk ekstrak etanol 65%, [BMIM]Br dan residu masing-masing yaitu 64,067 pg/mL, 16,063 pg/mL dan 111,245 pg/mL. Hasil penelitian dapat disimpulkan bahwa cairan ionik [BMIM]Br dapat menarik senyawa retrorsin JV-oksida lebih tinggi serta waktu ekstraksi yang lebih singkat, serta dapat menurunkan toksisitas residu secara in-vitro pada sel HepaRg.

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Comfrey (*Symphytum officinale* L.) contains allantoin, the phenolic compound rosmarinic acid, pyrrolizidine alkaloids and their derivatives, which have been used to treat fractures, tendon damage, ulceration in the digestive tract and pulmonary edema. The application of the ionic liquid extraction method by microwave was developed to obtain the bioactive content from comfrey leaves. The purpose of this study was to obtain the optimum comfrey leaf extraction conditions parameters to extract pyrrolizidine alkaloids

represented by retrorsin iV-oxide and allantoin compounds and evaluate their toxicity in vitro with HepaRg cells treated with optimum comfrey leaf extract. Comfrey leaves were extracted with 8 ionic liquids by microwave assisted extraction (MAE) and screening for total phenol, retrorsine Noxide and allantoin contents. The best ionic liquid from the screening was carried out by optimizing the response surface methodology (RSM) with the Box-Behnken design against four factors and three levels, which were used to determine the optimum extraction conditions for retrorsin iV[^]oxide and allantoin contents. The best optimized ionic liquid extract was evaluated for antioxidant activity of DPPH and ABTS, as well as in vitro toxicity tests with HepaRg cell line. The best optimized ionic liquid extract was evaluated for antioxidant activity of DPPH and ABTS. Toxicity tests in vitro used HepaRg cells. The results of the ionic liquid screening showed that [BMIM]Br was the best ionic liquid with a total phenol content of $0,01672 \pm 0,001$ mg/g powder, retrorsine iV-oxide $0,049 \pm 0,007$ mg/g powder, and allantoin $1,335 \pm 0,243$ mg/g powder. The results of the optimization of extraction from the ionic liquid [BMIM]Br obtained the highest levels of retrorsin A-oxide at run 24 with $40,653 \pm 0,002$ pg/g powder, and the highest allantoin content at run 27 with $1,282 \pm 0,0137$ mg/g powder. The results of the optimization of the extraction of ionic liquid [BMIM]Br gave optimum conditions at solvent concentration [BMIM]Br 1,16 mol/L, liquid/solid ratio 20,98 mL/g, power 45.09%, and extraction time 12,07 minutes, the levels of retrorsin A-oxide was 31,73 pg/g and allantoin was 0,809 mg/g. The antioxidant activity of both DPPH and ABTS showed the lowest values in the [BMIM]Br extract with IC₅₀ of 555,102 g/mL and 706,232 g/mL, respectively. The LC₅₀ values for ethanol 65% extract, [BMIM]Br and residues were 64,067 g/mL, 16.063 g/mL and 111,245 g/mL, respectively. The results of this study concluded that the ionic liquid [BMIM]Br can attract higher retrorsin iV-oxide compounds and shorter extraction times and can reduce residual toxicity in vitro in HepaRg cells.