OPTIMIZATION OF JACALIN EXTRACTED FROM JACKFRUIT (*Artocarpus heterophyllus*) SEED USING REVERSE MICELLAR EXTRACTION

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We hereby declare that we have checked this thesis, and in our opinion, this thesis is adequate in terms of scope and quality for the award of the degree of Doctor of Philosophy.

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I hereby declare that the work in this thesis is based on my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously or concurrently submitted for any other degree at Universiti Malaysia Pahang or any other institutions.

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ABSTRAK

Jakalin berpotensi tinggi dalam meningkatkan keberkesanan terapeutik ubat-ubatan, terutamanya dalam diagnosis and sasaran aktif sel-sel kanser. Namun, penggunaan secara meluas jakalin adalah masih terbatas disebabkan proses penulenan secara konvensional jakalin dari biji nangka yang mahal, mengambil masa dan sukar untuk ditingkatkan. Matlamat kajian ini adalah untuk mengkaji aplikasi pengekstrakan misel terbalik (RME) yang terdiri daripada misel terbalik sodium bis (2-ethylhexyl) sulfosuccinate (AOT) dalam isooktana untuk pengekstrakan dan penulenan jacalin daripada ektrak mentah biji nangka. Untuk pengoptimuman pengekstrakan hadapan, kaedah satu-faktor-pada-satu-masa (OFAT) dan reka bentuk pemfaktoran pecahan (FFD) digunakan untuk menentukan factor penting yang mempengaruhi kecekapan pengekstrakan hadapan (FEE). FFD menunjukkan bahawa pH larutan akuas, kepekatan AOT dan kepekatan NaCl merupakan factor penting dan telah dioptimumkan menggunakan reka bentuk Box-Behnken (BBD). Nilai maksimum FEE sebanyak 88.04 ± 1.30% dicapai pada keadaan optimum pH 4.58, NaCl 125 mM dan AOT 40 mM. Untuk pengekstrakan ke belakang, kesan pH larutan akuas pelucutan, kepekatan KCl dan isopropil alkohol (IPA) terhadap kecekapan pengekstrakan belakang (BEE) dinilai berdadarkan eksperimen OFAT diikuti dengan pengoptimuman menggunakan BBD. BEE maksimum sebanyak 92.60 ± 0.84% diperoleh pada pH 9.0, 0.75M KCl dan 40% v/v IPA. Kinetik pembahagian dan isoterma pada keadaan pengektrakan hadapan yang optimum turut dikaji menggunakan pseudo-order pertama (PFO), pseudo-order kedua (PSO), model Langmuir dan model Freundlich. Data kinetik pengekstrakan hadapan yang optimum didapati sesuai dengan model PSO (R^2 = 0.9829) berbanding model PFO (R^2 = 0.9738). Model isoterma Langmuir didapati paling sesuai berdasarkan nilai R^2 yang lebih tinggi (R2=0.9315) berbanding Freundlich (R2=0.8728). Mekanisme pemindahan jisim berdasarkan teori dua filem mendedahkan bahawa julat pekali pemindahan jisim keseluruhan untuk pengekstrakan hadapan ialah 0.2335×10^{-8} m^3/s hingga 0.7940×10^{-8} m^3/s untuk pengekstrakan hadapan dan 0.1852 $\times 10^{-8}$ m^3/s hingga 0.3479×10^{-8} m^3/s untuk pengekstrakan ke belakang. Untuk kesan pH, pengekstrakan ke hadapan dihadkan oleh penyerapan dalam fasa misel terbalik, manakala rintangan resapan dalam fasa pelucutan akuas mengawal pengektrakan ke belakang. Ujian toksisiti *in vivo* menggunakan embrio zebrafish mendapati bahawa ekstrak biji nangka dan ekstrak jakalin menghasilkan kesan toksik terhadap kecacatan dan perkembangan embrio. Nilai LC_{50} selepas terdedah selama 96 jam kepada ekstrak biji nangka ialah 20.48 µg/ml manakala bagi ekstrak jakalin ialah 14.81 µg/ml. Pelbagai kelainan perkembangan termasuk serta kesan teratogenik seperti edema perikardial, kelengkungan tulang belakang, penurunan kadar denyutan jantung dan kelewatan penetasan juga turut diperhatikan bergantung kepada kepekatan. Secara keseluruhan, penyelidikan ini menunjukkan bahawa sistem misel terbalik AOT/isooktana boleh digunakan sebagai proses yang berkesan untuk pengekstrakan dan penulenan jakalin daripada ekstrak biji nangka dengan kecekapan pengekstrakan maksimum. Jakalin yang diekstrak, bagaimanapun boleh mendatangkan bahaya sekiranya digunakan pada dos yang lebih tinggi terutamanya pada embrio. Pada masa hadapan, kaedah RME boleh dieksploitasi untuk pengektrakan dan penulenan protein yang bernilai tinggi daripada campuran biologi kompleks. Walau bagaimanapun, analisis ketoksikan terperinci ke atas protein yang diekstrak perlu dilakukan untuk memastikan keselamatannya terhadap embrio yang sedang berkembang.

ABSTRACT

Jacalin has excellent potentials in improving the therapeutic efficacy of anticancer drugs, especially in the diagnosis and active targeting of cancer cells. However, the widespread application was limited by the conventional purification process that was expensive, time-consuming and difficult to scale up. The aim of this study was to investigate the application of reverse micellar extraction (RME) consisting of sodium bis(2-ethylhexyl) sulfosuccinate (AOT) reverse micelles in isooctane for the recovery and purification of jacalin from the jackfruit seed crude extract. For the optimization of forward extraction, one-factor-at-a-time (OFAT) approach and fractional factorial design (FFD) was first employed to determine the significant factors affecting the forward extraction efficiency (FEE). The FFD determined that the aqueous phase pH, NaCl concentration and AOT concentration were the significant factors and were optimized using Box-Behnken design (BBD). The maximum FEE of 88.04±1.30% was obtained at aqueous phase pH 4.58, 125 mM NaCl and 40 mM AOT. For backward extraction, the effects of stripping aqueous phase pH, KCl concentration and isopropyl alcohol (IPA) on backward extraction efficiency (BEE) were evaluated based on OFAT experiments followed by optimization using BBD. The maximum BEE was 92.60 ± 0.84 % at pH 9.0, 0.75M KCl and 40 %v/v IPA. The partitioning kinetic and isotherm at the optimized conditions of forward extraction were examined using pseudo first order (PFO), pseudo second order (PSO), Langmuir and Freundlich models. The results revealed that the optimized forward extraction data fit well with the PSO model (R^2 = 0.9829) than the PFO model (R^2=0.9738). Isotherm studies indicated that the Langmuir isotherm model best fit the data with a high R^2 value (0.9315) than the Freundlich (R^2=0.8728). Mass transfer mechanism based upon the two-film theory revealed that the range of combined mass transfer coefficients obtained is 0.2335×10^{-8} m^3/s to 0.7940×10^{-8} m^3/s for forward extraction and 0.1852×10^{-8} m^3/s to 0.3479×10^{-8} m^3/s for backward extraction. For the effect of pH, the forward extraction was limited by the diffusion in the reverse micellar phase, while the diffusion resistance in the stripping aqueous phase controls the backward extraction. Zebrafish embryos treated with different concentration of the jackfruit seed crude extract and extracted jacalin showed embryonic and developmental defects. The 96hr-LC_{50} of jackfruit crude seed extract and jacalin were 20.48 µg/ml and 14.81 µg/ml, respectively. Multiple developmental abnormalities such as pericardial edema, spinal curvature, decreased heart rate and delayed hatching were observed in a concentration-dependent manner. Overall, this study indicated that the AOT/isooctane reverse micellar system is an efficient method to extract and purify jacalin from jackfruit seeds with maximum extraction efficiency. The extracted jacalin, however, could pose threats when consumed at higher doses especially on embryos. In the future, the RME method may be exploited for the recovery and purification of similarly high-value proteins from complex biological mixtures. However, detailed toxicity analysis on the extracted proteins should be performed to ascertain their safety on the developing embryos.

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