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EXTRACTION OF BIOACTIVE FLAVONOID COMPOUNDS FROM PECAH KACA (STROBILANTHES CRISPUS) USING SUPERCRITICAL CARBON DIOXIDE

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EXTRACTION OF BIOACTIVE FLAVONOIDS COMPOUNDS FROM PECAH KACA (STROBILANTHES CRISPUS) USING SUPERCritical CARBON DIOXIDE

By
LIZA BT MD SALLEH

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirements for the Degree of Doctor of Philosophy

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DEDICATION

To my dear husband, Izadin Tendot bin Saleh for his support and affectionate caring each moment in my life, especially throughout my study, specially dedicated to my kids Nurizzati Syaheerah, Muhammad Ezad Hakimi and Nur Batrisyia Maisarah, my late father Haji Md Salleh Bin Samad and my mother, Hajah Khadijah Bt Abd Manap for her spiritual support and doa, ‘terimakasih mak’, that I owe them each moment of my life.
EXTRACTION OF BIOACTIVE FLAVONOIDS COMPOUNDS FROM PECAH KACA (STROBLANTHES CRISPUS) USING SUPERCritical CARBON DIOxIDE

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February 2010

Chairman : Professor Russly Bin Abdul Rahman, PhD
Faculty : Engineering

Due to increasingly stringent environmental regulations, supercritical fluid extraction (SFE) has gained wide acceptance in recent years as an alternative to conventional solvent extraction for separation of organic compounds in many analytical and industrial processes. Consequently, supercritical fluid extraction (SFE) as an environmentally responsible and efficient extraction technique for solid materials was introduced and extensively studied for separation of active compounds from herbs and other plants. Supercritical carbon dioxide (SC-CO\textsubscript{2}) is one of widely used SCF. This is because of its mild critical conditions, non-toxic, non-explosive, readily available and easily removable from the products.

The extraction study of bioactive flavonoids using liquid organic solvents was carried out using the standard soxhlet method. From this study it found that ethanol was the best solvent for extraction of bioactive flavonoids, in terms of high extract and component yield. Analysis of the bioactive flavonoids compounds
extractable from *S. crispus* was accomplished by High Performance Liquid Chromatography (HPLC).

The first part of this study investigated the effects of pressure, temperature and dynamic extraction time as dependent variables of the SC-CO$_2$ extraction of bioactive flavonoid compounds. A full factorial in complete randomized design (CRD) was applied to obtain the best extraction conditions and mean value ($k$) between levels was calculated for different extraction conditions. From the $k$ value it can be concluded that pressure (with $k$ value of 11.79) had a dominant effect on the extraction yield after which followed by temperature (with $k$ value of 10.85) and dynamic time (with $k$ value of 1.53).

Based on a previous study it was found that pressure and temperature were the most important conditions in the SC-CO$_2$ extraction, therefore for the optimization of the extraction conditions, pressure and temperature with additional co-solvent (ethanol) flow rate were selected as the extraction variables. The Box Behnken Design (BBD) based on three factors and three levels was employed to obtain the optimum condition for SC-CO$_2$. Result showed that the optimum conditions were pressure at 200 bar, temperature at 50°C and co-solvent flow rate of 5 g/min respectively. Statistically, the yield was significantly affected by pressure and co-solvent flow-rate ($p<0.05$), however no significant showing was found for temperature.

The mathematical model was developed in order to estimate the correlation between experimental data and theoretical prediction for different parameters of SC-CO$_2$ namely CO$_2$ flow rate, pressure and temperature. The best extraction was obtained at
CO₂ flow rate of 15 g/min, pressure of 200 bar and temperature of 50°C. Under this condition, 6.87% of initial extraction yield of flavonoid compounds obtained in the leaves was extracted. The correlation between external mass transfer coefficient and the physical properties of supercritical carbon dioxide-ethanol mixture and leaves particles in term of dimensionless number was $Sh = 2.368 \, Re^{0.271} \, Sc^{0.33}$, which generates AARD of 2.67%. In general, the desorption model and Sovova model is better than adsorption equilibrium model for the estimation of extraction profile.
Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk Ijazah Doktor Falsafah

PENGEKSTRAKAN KOMPAUN BIOAKTIF FLAVONOID DARI PECAH KACA (STROBILANTHES CRISPUS) MENGGUNAKAN BENDALIR LAMPAU GENTING KARBON DIOKSIDA

Oleh

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Februari 2010

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Memandangkan terdapat peningkatan undang-undang alam sekitar yang ketat terhadap kaedah pengekstrakan, maka pada masa ini kaedah pengekstrakan bendalir lampau genting (SFE) telah mendapat sambutan yang menggalakan. Ia merupakan kaedah pengekstrakan alternatif selain dari pengekstrakan pelarut bagi memisahkan kompoun-kompaun organic samaada untuk proses analisis atau industri.

Oleh yang demikian, kaedah pengekstrakan SFE yang mesra alam dengan kaedah pengekstrakan berkesan ini telah digunapakai secara meluasnya dalam kajian bagi memisahkan kompaun-kompaun aktif didalam bahan pejal seperti herba dan tumbuh-tumbuhan. Bendalir lampau genting-karbon dioksida telah digunakan secara meluas sebagai bendalir lampau genting. Ini aadalah kerana sifat pada titik gentingnya yang sempurna, tidak beracun, tidak mudah terbakar dan sangat ekonomi. Kajian pengekstrakan komponen bioaktif flavonoid menggunakan pelarut organic dilakukan dengan kaedah pengekstrakan soxhlet.Daripada kajian ini didapati
ethanol merupakan pelarut yang terbaik bagi pengekstrakan diukur dari segi hasil dan jumlah kompoun terekstrak. Bagi mengenalpasti kompoun-kompoun terekstrak, penganalisaan adalah menggunakan kaedah kromatografi cecair berprestasi tinggi (HPLC).

Bahagian pertama kajian ini adalah mengkaji kesan tekanan, suhu dan masa pengekstrakan sebagai parameter bebas didalam SC-CO$_2$. ‘Full Factorial Design’ dengan Serakan Lengkap Design (CRD) telah digunakan bagi mendapatkan kondisi pengekstrakan terbaik. Nilai purata (K) diantara aras telah dikira bagi setiap kondisi pengekstrakan. Daripada nilai K, boleh disimpulkan bahawa tekanan (nilai K adalah 11.79) memberikan kesan yang paling berpengaruh terhadap hasil pengekstrakan, dan diikuti oleh suhu (nilai K adalah 10.85) dan masa pengekstrakan (nilai K adalah 1.53).

Berdasarkan keputusan kajian bahagian pertama, didapati bahawa tekanan dan suhu merupakan factor penting di dalam pengekstrakan SC-CO$_2$. Oleh yang demikian bagi kajian kondisi proses pengoptimuman, kedua-dua faktor tekan dan suhu diambilkira dan dengan penambahan kadar alir co-solvent ethanol. Reka bentuk Box Behnken (BBD) berdasarkan 3 faktor dan 3 aras telah digunakan untuk mendapatkan kondisi optimum bagi SC-CO$_2$. Keputusan menunjukan keadaan pengekstrakan optimum dicapai pada tekanan 200 bar, suhu 50°C dan kadar alir co-solvent adalah 50 g/min. Secara statistik, didapati bahawa hasil ekstrak adalah sangat dipengaruhi oleh tekanan dan kadar alir co-solvent (P< 0.05).
Model matematik dibangunkan bagi melihat perkaitan diaantara data eksperimen dengan penilaian secara teori. Oleh yang demikian tiga kaedah permodelan yang digunakan iaitu adsorption, desorption dan Sovova. Pengekstrakan terbaik adalah pada kondisi kadar alir carbon dioxide 15 g/min, tekanan 200 bar dan suhu 50°C dengan hasil 6.87%. Perkaitan diantara pekali pindah jisim luan dengan sifat fizikal camouran CO2-ethanol bagi daun tersebut dalam sebutan nombor sherwood adalah, \[ Sh = 2.368 \, Re^{0.271} \, Sc^{0.33} \]. Secara umumnya model desorption dan sovova adalah sesuai untuk menggambarkan profil pengekstrakan.
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First of all I would like to express my utmost thanks and gratitude to Almighty Allah s.w.t, the Sustainer, the most Gracious and most Merciful; without Whom will no one can achieve anything. My salawat and salam is addressed to His righteous messenger, prophet Muhammad s.a.w.

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Liza Bt Md Salleh

December 2009
I certify that a Thesis Examination Committee has met on 22 February 2010 to conduct the final examination of Liza Bt Md Salleh on her Phd thesis entitled “Extraction of Bioactive Flavonoid Compounds from Pecah Kaca (Strobilanthes crispus) using Supercritical Carbon Dioxide” in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The committee recommends that the candidate be awarded the Doctor of Philosophy.

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Date: 24 June 2010
This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for degree of Doctor of Philosophy. The members of Supervisory Committee were as follows:

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Date: 15 July 2010
DECLARATION

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

________________________
LIZA BT MD SALLEH

Date:
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### CHAPTER

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