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EXTRACTION OF VALUABLE COMPOUNDS FROM WINTER MELON (BENINCASA HISPIDA (THUNB.) COGN.) SEEDS USING ULTRASONICASSISTED SOLVENT, SUPERCRITICAL CARBON DIOXIDE AND PRESSURE-SWING METHODS

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BY

MANDANA BIMAKR

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December 2012

ESPECIALLY DEDICATED TO MY BELOVED FAMILY

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Doctor of Philosophy

EXTRACTION OF VALUABLE COMPOUNDS FROM WINTER MELON (*BENINCASA HISPIDA* (THUNB.) COGN.) SEEDS USING ULTRASONIC-ASSISTED SOLVENT, SUPERCRITICAL CARBON DIOXIDE AND PRESSURE-SWING METHODS

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Faculty: Food Science and Technology

Different extraction techniques have been used to isolate valuable compounds from different plants. Conventional Soxhlet extraction (CSE) is a standard technique which has been used for a long time. The main disadvantages of conventional techniques are long extraction time which is not always accepted by industries. This extraction method is also not suitable for the extraction of thermo-sensitive compounds due to the possibility of thermal decomposition of target compounds as extraction usually occurs at the boiling point of used solvent for a long time. The current study presented the effect of various extraction methods including conventional Soxhlet extraction (CSE), ultrasound-assisted extraction (UAE), supercritical carbon dioxide (CO_2) extraction (SCE) and SCE with pressure swing technique (SCE-PST) for extraction of valuable compounds from winter melon (*Benincasa hispida* (Thunb.) Cogn.) seeds. Parameters examined were crude extraction yield (CEY), radical scavenging activity (RSA) in terms of 1, 1-diphenyl-

2-picrylhydrazyl (DPPH˙) radicals scavenging percentage (%$DPPH_{sc}$) and 2,2´-Azinobis (3-ethylbenzothiazoline-6-sulphonic acid) diammonium salt ($ABTS^{+\cdot}$) radicals scavenging percentage (%$ABTS_{sc}$) and total phenolic content (TPC) of *B. hispida* seeds. The overall optimum conditions for obtaining maximum dependent variables were predicted using response surface methodology (RSM). Under the optimised conditions, the fatty acid composition of extracts was identified and quantified using gas chromatography (GC). In CSE different solvents (*n*-hexane, ethanol and ethyl acetate) were used and the highest CEY (294.45 ± 1.22 mgg^{-1}) obtained using ethyl acetate. Ethanol (EtOH) extracts showed the highest %$DPPH_{sc}$ and %$ABTS_{sc}$ (28.7 ± 0.7 and 27.0 ± 0.9, respectively) while *n*-hexane extracts possessed the lowest (13.1 ± 0.9 and 12.2 ± 0.6, respectively).

TPC ranging from zero in *n*-hexane extract to 11.34 ± 1.3 mg GAE/g extracts obtained using EtOH which revealed the antioxidant potency of *B. hispida* seeds. In order to improve the quality and quantity of the extracts, UAE was applied and significant (p<0.05) effect of amplitude, temperature and sonication time was detected. The overall optimum conditions predicted to be 65% amplitude, 52 ºC and 35.00 min. under the optimum conditions the CEY, %$DPPH_{sc}$, %$ABTS_{sc}$ and TPC were 108.50 mgg^{-1}, 43.47%, 36.47% and 23.97 mg GAE/g extract. In the following, the SCE was investigated and it was found that studied parameters (pressure, temperature and dynamic extraction time) significantly (p<0.05) affected the dependent variables. The optimum conditions found to be 234.25 bar, 46 ºC and 95.05 min and under this optimum condition, the CEY, %$DPPH_{sc}$, %$ABTS_{sc}$ and TPC were 174.91 mgg^{-1}, 52.96%, 61.65% and 40.45 mg GAE/g extract, respectively. Finally, SCE-PST was investigated at different levels of pressure, holding time and continuous extraction time. The significant (p<0.05) difference in

responses revealed the crucial and effective role of PST for improving the SCE process. The overall optimum conditions predicted to be 179.18 bar, 10.15 min holding time and 52.47 min continuous extraction time. Under the optimum condition the CEY, $\%DPPH_{sc}$, $\%ABTS_{sc}$ and TPC were 235.12 mgg^{-1}, 67.36%, 64.42% and 42.77 mg GAE/g extract, respectively. Significant difference ($p<0.05$) for fatty acid composition was found between CSE and various extraction methods while considerable difference is not found between the unsaturated fatty acids (UFAs) content of extracts using UAE (82.00%), SCE (83.67%) and SCE-PST (84.34%). Generally, the dominant composition of the UFAs was linoleic acid (C18:02) and oleic acid (C18:01). This study, for the first time, confirmed the possibility of using UAE, SCE and SCE-PST to obtain the valuable extracts with high antioxidant potency and containing high amount of UFAs from *B. hispida* seeds.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

PENGEKSTRAKAN KOMPAUN BERKUALITI DARIPADA BIJI KUNDUR (*BENINCASA HISPIDA* (THUNB.) COGN.) MENGGUNAKAN KAEDAH PENGEKSTRAKAN BENDALIR GENTING LAMPAU KARBON DIOKSIDA DAN KAEDAH-KAEDAH PENGEKSTRAKAN LAIN

Oleh

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Kaedah pengekstrakan berbeza telah digunakan untuk mengasingkan kompaun berkualiti dari pelbagai jenis tumbuhan berbeza. pengekstrakan konvensional Soxhlet (CSE) merupakan teknik standard yang telah digunakan sekian lama. Walaubagaimanapun, kelemahan utama teknik ini adalah masa pengekstrakan yang panjang yang tidak diterima di industri. Kedah pengekstrakan ini juga tidak sesuai untuk pengekstrakan sebatian atau kompaun yang sensitive terhadap kepanasan. Ini kerana pengekstrakan dilakukan pada suhu tinggi (takat didih pelarut) pada masa yang lama yang akan mengakibatkan kemungkinan berlakunya penguraian terma terhadap kompaun yang ingin diekstrak.

Kajian yang dijalankan membentangkan kesan pelbagai kaedah pengekstrakan seperti pengekstrakan konvensional Soxhlet (CSE), pengekstrakan ultra sound terbantu (UAE), pengekstrakan bendalir genting lampau CO_2 (SCE) dan pengekstrakan SCE teknik tekanan berayun (SCE-PST) bagi mengekstrak kompaun

bernilai daripada biji kundur (*Benincasa hispida* (Thunb.) Cogn.). pemboleh ubah yang diukur adalah hasil pengekstrakan (CEY), aktiviti memerangkap radikal (RSA) berlandaskan 1, 1-diphenyl-2-picrylhydrazyl ($DPPH^{\bullet}$) peratus pemerangkapan radikal ($\%DPPH_{sc}$) dan 2,2´-Azinobis (3-ethylbenzothiazoline-6-sulphonic acid) peratus pemerangkapan radikal ($\%ABTS_{sc}$) garam ammonium ($ABTS^{+\bullet}$) dan total kandungan phenol (TPC) daripada biji kundur (*B. hispida*). Kondisi optimum keseluruhan, untuk mendapatkan pemboleh ubah bersandar maksimum diramalkan dengan menggunakan kaedah rangsangan permukaan (RSM). Pada nilai optimum, komposisi asid lemak yang diekstrak dikenalpasti dan dikira menggunakan kromatogrofi gas (GC). Bagi CSE, pelarut yang berbeza digunakan (*n*-hexane, ethanol, etil asetat) dimana penghasilan ekstrak tertinggi diperoleh dengan menggunakan etil asetat (294.45 ± 1.22 mgg^{-1}). Ekstrak ethanol (EtOH) pula memberikan, nilai paling tinggi pada $DPPH^{\cdot}$ dan $ABTS^{\cdot+}$ (28.70 ± 0.70 dan 27.00 ± 0.90%) kebolehan mengikat radikal bebas, manakala pengektrakan menggunakan *n*-hexane menghasilkan nilai terendah (1.31 ± 0.90 dan 12.20 ± 0.60% bagi $DPPH^{\cdot}$ dan $ABTS^{\cdot+}$). Nilai TPC daripada julat kosong dengan menggunakan ekstrak *n*-hexane hingga 11.34 ± 1.33 mg GAE/g dengan menggunakan ekstrak etanol, menunjukkan potensi biji kundur (*B. hispida*) berupaya bertindak sebagi antioksida. Bagi meningkatkan kualiti dan kuantiti ekstrak, UAE telah digunakan dan kesan ketara ($p<0.05$) bagi amplitude, suhu dan masa sonikasi dikesan. Nilai kondisi purata optimum telah diramalkan pada 65%, 52 °C dan 35 min yang menghasilkan 108.50 mgg^{-1} CEY, 43.47 $\%ABTS_{sc}$, 36.47 $\%DPPH_{sc}$ dan 23.97 mg GAE/g ekstrak. Seterusnys adalah kajian terhadap SCE dan didapati parameter yang dikaji (tekanan, suhu dan masa pengekstrakan dinamik) secara umumnya telah memberi kesan ketara ($p<0.05$) terhadap pemboleh ubah bersandar. Pada kondisi optimum (234.5 bar, 46

°C dan 95.05 min) CEY, %$ABTS_{sc}$, %$DPPH_{sc}$ dan TPC adalah masing-masing 174.91 mgg^{-1}, 52.96%, 61.65% dan 40.45 mg GAE/g ekstrak. Akhir sekali, SCE-PST telah dikaji pada tekanan, masa statik dan masa pengekstrakan berterusan yang berbeza. Rangsangan ketara (p<0.05) menunjukkan kesan efektif PST dalam penambahbaikan proses SCE. Kondisi optimum keselurahan diramalkan pada 179.18 bar, 10.15 min bagi masa statik dan 52.47 minit bagi proses berterusan. Pada nilai optimum, CEY, %$ABTS_{sc}$, %$DPPH_{sc}$ dan TPC adalah masing-masing 235.12 mgg^{-1}, 67.36%, 64.42% dan 42.77 mg GAE/g ekstrak. Perbezaan ketara (p<0.05) bagi komposisi asid lemak adalah diperoleh dari pada CSE dibandingkan dengan kaedah pengekstrakan yang lain manakala tiada perbezaan ketara pada kandungan asid lemak tak tepu (UFAs) menggunakan UAE (82.00%), SCE (83.67%) dan SCE-PST (84.34%). Secara umumnya komposisi dominan bagi UFAs adalah asid lenolik (C18:02) dan asid oleik (C18:01). Kajian ini untuk pertama kalinya mengesahkan kemungkinan menggunakan UAE, SCE dan SCE-PST bagi memperolehi bahan berguna, dengan kandungan bahan antioksida poten serta UFA dengan amaun tinggi hasil pengekstrakan biji kundur (*B. hispida*) ini.

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I certify that an Examination committee met on _ to conduct the final examination of Mandana Bimakr on her Doctor of Philosophy thesis entitled "Extraction of valuable compounds from winter melon (*Benincasa hispida*) seeds using supercritical carbon dioxide and other extraction methods" in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and University Pertanian Malaysia (Higher Degree) Regulation 1981. The committee recommends that the candidate be awarded the relevant degree. Members of Examination Committee are as follows:

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DECLARATION

I declare that the thesis is 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 Putra Malaysia or at other institutions.

_____

MANDANA BIMAKR

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