UNIVERSITI PUTRA MALAYSIA

EXTRACTION OF TOTAL PHENOLIC AND TOTAL MONOMERIC ANTHOCYANIN CONTENTS FROM MANGOSTEEN (GARCINIA MANGOSTANA L.) HULL

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By

CHEOK CHOON YOONG

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

July 2012

DEDICATED TO

my beloved father who passed away on 29 October 2008. I am thankful and grateful for his sacrifices and determination to enable his children to get an education by emphasizing the Chinese proverb "Poor in wealth but rich in education". My motivation in pursuance of this PhD program comes from his determination. He always felt proud of his children's enthusiasm in pursuing further education degrees and acquiring new knowledge. In further appreciation of his sacrifices, I selected mangosteen, which was cultivated by him, as a sample source for this PhD research.

and to my sister, who passed away on 3 November 2012. She was also my mathematic and English teacher.

In memory

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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 TOTAL PHENOLIC AND TOTAL MONOMERIC ANTHOCYANIN CONTENTS FROM MANGOSTEEN (*GARCINIA MANGOSTANA* L.) HULL

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Chairman : Chin Nyuk Ling, PhD

Faculty : Engineering

Mangosteen is a popular local tropical fruit because the pulp is delicious and has high value of nutrient and mineral content. However, mangosteen hull is disposed of as waste by local people due to the unpleasant taste of bitterness, though it contains total phenolic and anthocyanins which have numerous important pharmaceutical properties. Due to the remarkable relationship between total phenolic content (TPC) and total monomeric anthocyanin (TMA) with antioxidant capacity, a series of investigations were undertaken to enhance the mangosteen crude extract yields evaluated in terms of TPC and TMA. This research involved investigations from fundamental to optimization, based on hypotheses of methanol, acidic condition of extraction solvent, and the ultrasonic wave treatment could give higher extraction yield. Results were expressed in mg gallic acid/g hull powder for TPC, while for TMA, expressed in mg cy-3-glu/g hull powder.

The effect of solvents, i.e. ethyl acetate, isopropanol, acetone, ethanol, methanol, and distilled water were evaluated on the extraction of total phenolic content (TPC) from

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mangosteen. Methanol was found as the most effective solvent in giving the highest recovery ($p < 0.05$) of TPC, compared to ethanol, acetone, isopropanol, ethyl acetate and distilled water. Regression equations of strong coefficients of determinations ($R^2 \geq 0.990$) were obtained for UV-Vis spectrophotometer direct absorbance of acetone, ethanol, and methanol extracts with the Folin-Ciocalteu (FC) method, suggesting regression equations obtained can be used to determine TPC without using the FC method.

The optimum conditions of extraction time, solid to solvent ratio, and methanol concentration for extraction of total phenolics from mangosteen hull powder was investigated using the response surface methodology (RSM). The experimental data obtained was adequately fitted into second-order polynomial models with R^2 of 0.897. Optimal extraction parameters which gave a maximum TPC yield of 140.66 mg (gallic acid equivalent) GAE/g powder were from a 2 hours' extraction with 0.05 solid to solvent ratio and at 69.77% methanol concentration. Analysis using artificial neural network (ANN) predicted data which showed a higher R^2 value of 0.945 and average absolute deviation values of 4.01% versus 5.37% for the RSM. This suggests that ANN is a better modeling technique for nonlinear data for predicting TPC yield extracted from mangosteen hull powder compared to the RSM.

The recovery of anthocyanins from mangosteen hull was investigated using aqueous methanol solvent acidified by juice from Mexican lime (*C. aurantifolia* Swingle) in comparison with the conventional HCl acidified methanol solvent. The addition of 0.20 ml lime juice/ml methanol aqueous extraction solvent gave a maximum total monomeric anthocyanin (TMA) recovery of 4.742±0.590 mg cy-3-glu/g hull powder

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compared to 2.950±0.265 when using HCl acidified extraction solvent at 0.20%. A significant increase of 60.75% ($p < 0.05$) anthocyanins recovery suggests that this natural Mexican lime juice can be a good acidifying agent and replaces HCl. Particle size hull powder of 250 μm to 500 μm was also found to give the highest recovery of anthocyanins ($p < 0.05$).

Two types of extraction solvents, i.e., methanol and ethanol aqueous solvent acidified with 0.20% HCl were used to investigate the effect of ultrasonication time and amplitude on TMA and TPC from mangosteen hull. The highest TMA recovery of 2.917±0.039 mg cy-3-glu/g hull powder was achieved using methanol 0.20% HCl acidified aqueous solvent with a pretreatment of an ultrasonic probe of 15 minutes' sonication time and 20% amplitude. While the highest recovery of TPC of 245.780±9.858 mg GAE/g hull powder was obtained using ethanol 0.20% HCl acidified aqueous solvent by pretreating the mangosteen sample with ultrasonic probe conditions of 25 minutes sonication time and 80% amplitude.

In conclusion, methanol has been found to be the best extraction solvent in giving the highest TPC yield. The optimum extraction conditions of 2 hours extraction time, 0.05 solid to solvent ratio and 69.77% methanol concentration obtained from RSM gave the maximum TPC yield 140.66 mg GAE/g hull powder with an improvement of 85.41% compared to extraction with 20 hours extraction time, 0.20 solid to solvent ratio and pure methanol solvent. The extraction solvent acidified with 20 ml of Mexican lime juice gave higher TMA yield compared to HCl acidified's. The ultrasonication of 15 minutes and 20% amplitude gave the highest TMA recovery of 2.917±0.039 mg cy-3-glu/g hull powder using acidified methanol solvent, while 25 minutes and 80%

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amplitude gave the highest TPC of 245.780±9.858 mg GAE/g hull powder using acidified ethanol solvent. Overall, the extraction of TPC and TMA yields from mangosteen hull have been optimized by reducing the extraction time, solid to solvent ratio using acidified extraction solvent with ultrasonic wave treatment.

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

PENGEKSTRAKAN KANDUNGAN JUMLAH FENOL DAN JUMLAH MONOMERIK ANTOSIANIN DARIPADA KULIT MANGGIS (*GARCINIA MANGOSTANA* L.)

Oleh

CHEOK CHOON YOONG

Julai 2012

Pengerusi : Chin Nyuk Ling, PhD

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Manggis ialah buah-buahan tropika tempatan yang popular kerana isinya adalah lazat dan mempunyai nilai zat dan mineral yang tinggi. Walau bagaimanapun, manggis kulit dibuangkan sebagai bahan buangan oleh orang-orang tempatan kerana rasa kepahitan yang tidak menyenangkan, walaupun ia terkandung jumlah fenolik dan antosianin yang mempunyai banyak sifat farmaseutikal yang penting. Disebabkan hubungan antara kandungan jumlah fenol (TPC) dan antosianin (TMA) dengan kapasiti antioksidan yang ketara, satu siri eksperimen telah dijalankan untuk mempertingkatkan hasil ekstrak manggis mentah yang dinilai dari segi kandungan jumlah fenol (TPC) dan antosianin (TMA). Kajian ini melibatkan eksperimen dari asas ke pengoptimuman, berdasarkan hipotesis bahawa metanol, pelarut berasid, dan rawatan gelombang ultrasonik boleh memperolehi hasil pengekstrakan yang lebih tinggi. Keputusan dinyatakan dalam mg asid gallic/g serbuk kulit untuk TPC, manakala bagi TMA, dinyatakan dalam mg cy-3-glu/g serbuk kulit.

Kesan pelarut, i.e. etil asetat, isopropanol, aseton, etanol, metanol dan air suling dikaji terhadap pengekstrakan jumlah kandungan fenol (TPC) daripada kulit manggis. Metanol

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telah didapati sebagai pelarut yang paling berkesan dalam memberi pulangan tertinggi ($p < 0.05$) TPC, berbanding dengan etanol, aseton, isopropanol, etil asetat dan air suling. Persamaan regresi dengan pekali penentuan yang tinggi ($R^2 \geq 0.990$) telah diperolehi secara langsung daripada serapan UV-Vis spektrofotometer untuk ekstrak aseton, etanol, dan metanol dengan kaedah Folin-Ciocalteu (FC), menunjukkan persamaan regresi yang diperolehi ini boleh digunakan untuk menentukan TPC tanpa menggunakan kaedah FC.

Keadaan optimum iaitu masa pengekstrakan, nisbah pepejal kepada pelarut, dan kepekatan metanol untuk pengekstrakan jumlah fenol daripada serbuk kulit manggis telah dikaji dengan menggunakan kaedah gerak balas permukaan (RSM). Data eksperimen yang diperolehi diisikan ke dalam model polinomial tertib kedua dengan R^2 0.897. Parameter pengekstrakan optimum yang memberi hasil TPC yang maksimum iaitu 140.66 mg GAE/g serbuk kulit adalah dari keadaan pengekstrakan 2 jam, 0.05 nisbah pepejal kepada pelarut dan kepekatan metanol pada 69.77%. Analisis menggunakan rangkaian neural tiruan (ANN) meramalkan data menunjukkan nilai R^2 yang lebih tinggi iaitu 0.945 dan nilai purata sisihan mutlak 4.01% berbanding 5.37% bagi RSM. Ini mencadangkan bahawa ANN adalah teknik pemodelan yang lebih baik berbanding dengan RSM bagi data tak linear dalam ramalan hasil TPC yang diekstrakkan daripada serbuk kulit manggis.

Pemulihan antosianin daripada kulit manggis telah dikaji menggunakan pelarut akueus berasid dengan jus limau nipis (*C. aurantifolia* Swingle) berbanding dengan pelarut tradisi iaitu metanol berasid HCl. Dengan tambahan 0.20 ml jus limau nipis/ml pengekstrakan mengunakan pelarut metanol akueus memberikan maksimum jumlah monomerik antosianin (TMA) sebanyak 4.742±0.590 mg cy-3-glu/g serbuk kulit berbanding dengan 2.950±0.265 mg cy-3-glu/g serbuk kulit apabila menggunakan

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pelarut pengekstrakan yang berasid dengan 0.20% HCl. Peningkatan yang ketara ($p < 0.05$) antosianin sebanyak 60.75% mencadangkan bahawa jus limau nipis semulajadi ini adalah ejen asid yang baik dan boleh menggantikan HCl dalam mengasidkan pelarut. Serbuk kulit manggis dengan saiz partikel antara 250 μm hingga 500 μm juga didapati memberi pemulihan antosianin tertinggi ($p < 0.05$).

Dua jenis pelarut pengekstrakan, iaitu, pelarut metanol dan etanol akueus berasid dengan 0.20% HCl telah digunakan untuk mengkaji kesan masa ultrasonik dan amplitud terhadap TMA dan TPC daripada kulit manggis. Pulangan TMA tertinggi sejumlah 2.917±0.039 mg cy-3-glu/g serbuk kulit telah dicapai dengan menggunakan pelarut metanol akueus berasid dengan 0.20% HCl dengan ultrasonik prapengolahan selama 15 minit dan 20% amplitud manakala pulangan TPC tertinggi sebanyak 245.780±9.858 mg GAE/g serbuk kulit telah diperolehi menggunakan pelarut etanol akueus berasid dengan 0.20% HCl selama 25 minit dan 80% amplitud.

Kesimpulannya, metanol telah didapati sebagai pelarut pengekstrakan yang terbaik dalam memberikan hasil TPC tertinggi. Keadaan pengekstrakan optimum 2 jam masa pengekstrakan, 0.05 nisbah pepejal kepada pelarut dan 69.77% kepekatan metanol diperolehi dari RSM memberi hasil TPC maksimum 140,66 mg GAE/g serbuk kulit dengan peningkatan sebanyak 85.41% berbanding pengekstrakan dengan 20 jam masa pengekstrakan, 0.20 nisbah pepejal kepada pelarut dan metanol pelarut tulen. Pelarut pengekstrakan berasid dengan 20 ml jus limau nipis memberi hasil TMA yang lebih tinggi berbanding dengan HCl berasid. Ultrasonikasi 15 minit dan 20% amplitud memberi pulangan TMA tertinggi 2.917±0.039 mg cy-3-glu/g serbuk kulit apabila menggunakan pelarut metanol berasid, manakala 25 minit dan 80% amplitud memberi pulangan TPC tertinggi 245.780±9.858 mg GAE/g serbuk kulit apabila menggunakan

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pelarut etanol berasid. Secara keseluruhan, pengekstrakan hasil TPC dan TMA dari manggis hull telah dioptimumkan dengan mengurangkan masa pengekstrakan, nisbah pepejal kepada pelarut menggunakan pelarut pengekstrakan berasid dengan rawatan gelombang ultrasonik.

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I certify that an Examination Committee has met on **19 July 2012** to conduct the final examination of Cheok Choon Yoong her thesis entitled "Extraction of Total Phenolic Content and Total Monomeric Anthocyanin from Mangosteen (*Garcinia Mangostana* L.) Hull" in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulations 1981. The Committee recommends that the student be awarded the Doctor of Philosophy.

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This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of **Doctor of Philosophy**.

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Date: 13 December 2012

DECLARATION

I hereby declare that the 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 UPM or other institutions.

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CHEOK CHOON YOONG

Date: 19 July 2012

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