



UNIVERSITI PUTRA MALAYSIA

***OPTIMIZATION OF PROTEIN EXTRACTION PROTOCOLS FOR THE
IDENTIFICATION OF ABUNDANTLY EXPRESSED PROTEINS IN THE
FRUIT, ROOT AND LEAF OF CURCULIGO LATIFOLIA***

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IDENTIFICATION OF ABUNDANTLY EXPRESSED PROTEINS IN THE
FRUIT, ROOT AND LEAF OF *CURCULIGO LATIFOLIA***

By

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Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia in
Fulfillment of the Requirement for the Degree of Master of Science

February 2012

Dedicated to:

My Father and Mother,

Mr. Mahmoud Rastegari

Madam Fatemeh Pazhoumand

My Beloved sister,

Mandana

Whoever has provided me with care
and compassion throughout my life

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfillment of the requirement for the degree of Master of Science

OPTIMIZATION OF PROTEIN EXTRACTION PROTOCOLS FOR THE IDENTIFICATION OF ABUNDANTLY EXPRESSED PROTEINS IN THE FRUIT, ROOT AND LEAF OF *CURCULIGO LATIFOLIA*

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Substantial achievements in proteomic techniques in the last decade have led to an increase in the application of proteomics to many fields, including plant sciences. Proteomics is a robust technology for the high-throughput investigation of proteins on a genome-wide scale. With the completion of genome sequencing projects and the improvement of analytical methods for protein identification, proteomics has now become a major tool for studying functional genomics. Despite the progress in proteomics research in animals, yeast and *Escherichia coli*, plant proteomics is still at infancy stages. Lemba, *Curculigo latifolia*, is an indigenous plant of Malaysia and is found mainly in swampy areas of tropical Asia and Australia. To date, there are no published reports of the proteome of this plant. This study focused on finding the best protein extraction protocols for specific tissues from this plant as

judged by the optimal 2-DE patterns. This was an essential step that had to be performed before the identification of abundantly expressed proteins from the fruit, root and leaf could be attempted. Fruit, root and leaf tissues of *Curculigo latifolia* were individually subjected to 7 different published protocols (three different phenol based protocols, a TCA-acetone based protocol, a combination of phenol and TCA-acetone-based protocol, and a chloroform-based protocol) to determine the most efficient method for the specific tissue. Prior to the protein extraction of fruit, a novel pectolytic enzyme mix pretreatment was tested and was found to successfully reduce the amount of pectin which otherwise made protein extraction from that tissue nearly impossible. Chloroform, phenol and TCA-acetone-based protocols were found to be the most efficient methods for fruit, root and leaf respectively. The five most abundant spots for each tissue were excised from 2-DE gels and analyzed by MALDI-TOF/TOF mass spectrometry, and identified by cross species comparisons employing available plant protein databases. Glutelin and RuBisCo were the most abundant proteins in the fruit and leaf respectively. In root, catalase and glyceraldehyde-3-phosphate were found to be the most abundant proteins.

Keywords: *Curculigo latifolia*, fruit, root, leaf, protein extraction, 2-D electrophoresis

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk Ijazah Master Sains

**PENGOPTIMUMAN PROTOKOL PENGEKSTRAKAN UNTUK PENENTUAN
PROTEIN YANG BAHYAK
DI EKSPRESKAH DATU AKAR, BUAH, DAN DAUN *CURCULIGO LATIFOLIA***

Oleh

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Pelbagai pencapaian dalam teknik proteomik beberapa dekad lepas telah mendorong kepada peningkatan dalam aplikasi proteomik dalam banyak bidang, termasuk sains tumbuhan. Proteomik adalah teknologi mantap yang berupaya melakukan pengenalpastian protein dalam jumlah yang tinggi pada skala genom. Dengan lengkapnya projek rangkaian genom dan penambahbaikan kaedah analitikal untuk pengenalpastian protein, proteomik akan menjadi alat utama untuk mengkaji fungsi genomik. Walaupun terdapat banyak perkembangan dalam kajian proteomik pada binatang, yis dan *Escherichia coli*, proteomik tumbuhan masih berada pada peringkat awal. Lemba, *Curculigo latifolia*, adalah tumbuhan asli Malaysia dan boleh ditemui juga di kawasan berpayau Australia dan Asia tropika. Sehingga kini, belum terdapat laporan

proteome diterbitkan tentang tumbuhan ini. Kajian ini memfokuskan pada pencarian dan penambahbaikan protokol pengekstrakan protein yang terbaik bagi tisu spesifik dari tumbuhan ini yang ditentukan daripada corak optimum 2-DE. Langkah ini adalah penting untuk dilakukan sebelum pengesanan protein yang banyak diekspreskan daripada buah, akar dan daun. Tisu daripada buah, akar dan daun *Curculigo latifolia* secara individunya diekstrak mengikut 7 protokol yang telah diterbitkan (3 protokol berasaskan fenol yang berbeza, protokol berasaskan TCA-aseton, kombinasi protokol menggunakan fenol dan TCA-aseton dan protokol berasaskan kloroform) untuk menentukan kaedah pengekstrakan paling efisien bagi tisu spesifik Lembu. Sebelum pengekstrakan protein pada buah dilakukan, enzim pektolitik telah ditambah sebagai rawatan awal dan didapati langkah ini berjaya mengurangkan amaun pektin yang menghalang kejayaan proses pengekstrakan protein tersebut. Kloroform, fenol dan protokol berasaskan aseton didapati antara kaedah paling efisien bagi buah, akar dan daun masing masing. Lima tempok paling banyak didapati untuk setiap tisu telah dipotong daripada gel 2-DE dan dianalisa menggunakan spektrometri jisim MALDI-TOF/TOF, dan dikenalpasti dengan perbandingan spesies rentas menggunakan pengkalan data protein tumbuhan sediaada. Glutelin dan RuBisCo merupakan protein yang paling banyak didapati masing-masing dalam buah dan daun. Pada akar, catalas dan gliseraldehid-3-fosfat merupakan protein yang paling banyak didapati.

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I certify that a Thesis Examination Committee has met on 17th of February 2012 to conduct the final examination of Elham Rastegari on her thesis entitled "Optimization of Protein Extraction Protocols for the Identification of Abundantly Expressed Proteins in the Fruit, Root and Leaf of *Curculigo latifolia*" 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 student be awarded the Master of Science.

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DECLARATION

I certify that the thesis is my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously, and is not currently submitted for any of other degree at University Putra Malaysia or at any other institutions.



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