



UNIVERSITI PUTRA MALAYSIA

**ANTIOXIDANT AND FLAVONOID ACTIVITIES IN DIFFERENT VARIETIES OF
FICUS DELTOIDEA JACK (MAS COTEK) LEAVES**

MOHD HAKIMAN MANSOR

FBSB 2013 30



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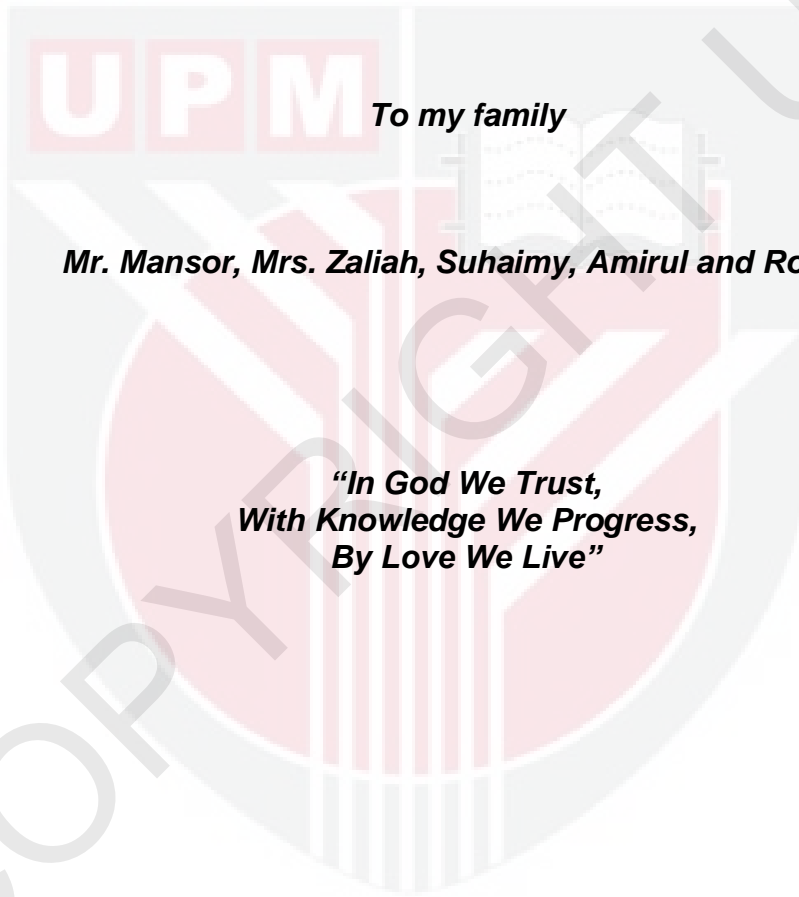
**Thesis submitted to the School of Graduate Studies, Universiti Putra
Malaysia, in Fulfillment of the Requirement for the Degree of Doctor of
Philosophy**

September 2013

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To my family

Mr. Mansor, Mrs. Zaliah, Suhaimy, Amirul and Rodiah

*“In God We Trust,
With Knowledge We Progress,
By Love We Live”*

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Abstract of thesis submitted to the Senate of Universiti Putra Malaysia in fulfillment of the requirement for the degree of Doctor of Philosophy

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September 2013

Chairman: Professor Maziah Mahmood, PhD

Faculty: Biotechnology and Biomolecular Sciences

Ficus deltoidea Jack or known as Mas Cotek in Malaysia is one of the most promising medicinal plants to be commercialised due to its high antioxidant activities. To date, there is no information on the best variety of *F. deltoidea* in terms of antioxidant activity for commercialisation purposes. Hence, the general objective of this study was to evaluate the antioxidant activity and flavonoid contents of three *F. deltoidea* varieties that are commonly used as herbal medicines in Malaysia. Specific objectives were to determine the total antioxidant capacity in leaf extracts of *F. deltoidea* using different solvent extractions, to analyse and identify the flavonoid content in leaf extracts of *F. deltoidea*, and to examine the flavonoid production in cell suspension culture of *F. deltoidea*. Two accessions from each variety were chosen. They were FDK1 and FDK2 (var. *kunstleri*), FDT1 and FDT2 (var. *trengganuensis*), and FDA1 and FDA2 (var. *angustifolia*). From this study, leaf extracts of *F. deltoidea* showed different activity depending on methods and solvents used. Leaf extracts from var. *trengganuensis* showed the highest total protein and reducing sugar content with 30.6 and 17.9 mg/g FW in FDT2 and FDT1, respectively while total hydrolysed sugar content was highest in leaf extract of FDA2 with 30.7 mg/g FW. Total ascorbic acid content was highest in leaf extract of FDT1 with 4.12 mg/g FW. Enzymatic antioxidant activity was highest in leaf extract of FDK1 for determination of catalase, peroxidase, ascorbate peroxidase and ascorbate oxidase activity with 0.23, 0.75, 9.01 and 23.20 unit/mg protein. Aqueous extract at room temperature (25 °C) of FDK1 showed the highest activity of total antioxidant with 1.85 mg Trolox equivalent (TE)/g fresh weight (FW) using Ferric

Reducing Antioxidant Potential (FRAP) method and leaf extract of FDT2 contained the highest total antioxidant using 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging method with 4.85 mg TE/g FW. Analysis of total antioxidant and phenolic compounds using hot and cold aqueous extracts of leaf extracts of *F. deltoidea* was showed that most of leaf extracts contained higher antioxidant content in hot aqueous extracts compared to that of cold aqueous extract. Furthermore, methanol and ethanol extracts of *F. deltoidea* were also analysed in which ethanol extracts showed more antioxidants as compared to that of methanol extracts. Leaf extract of *F. deltoidea* var. *kunstleri* (FDK1) contained the highest activity of total polyphenol content for both methanol and ethanol extracts with 1.12 and 1.13 mg GAE/g FW, respectively. The highest total phenolic acid content was found in leaf extract of accession FDK1 using methanol extraction with 4.54 mg GAE/g FW while from ethanol extraction, leaf extract of FDK2 showed the highest activity with 4.00 mg GAE/g FW. All six accessions, which represented three varieties, were analysed for specific flavonoid content using high performance liquid chromatography (HPLC). From the chromatograms, two flavonoid compounds; rutin and naringin were detected in all the intact plants where rutin was more abundant than naringin. Leaf extract of FDK1 showed the highest rutin content with 12.83 µg/g DW, while FDT2 contained the highest naringin content with 3.04 µg/g DW. Cell suspension culture was established from leaf-derived callus of accession FDK1. Cell suspension culture was initiated by culturing the callus liquid MSB5 basal media on an orbital shaker at 120 rpm. Cell suspension of FDK1 at day 12 was chosen for subsequent experiments due to its highest flavonoid production with 2.10 mg RE/g DW. An initial inoculum size of 2 g/culture was found to produce the highest biomass with 0.65 g/25 mL of media while the highest flavonoid production was found with an initial inoculum size of 0.5 g/culture with 3.3 mg RE/g DW. The biomass and flavonoid production of cell suspension culture of FDK1 was proportional with cell aggregate size with 0.28 g/25 mL of media and 3.3 mg RE/g DW, respectively using 750 µm mesh size compared to that of other cell aggregate sizes. Initial pH value of control (pH 5.75) was found to be the best for biomass and flavonoid production with 0.28 g/25 mL of media and 3.3 mg RE/g DW. Total antioxidant content of intact plant using DPPH method was 3-folds higher compared to that of cell suspension culture while 6-folds for FRAP method. At the same time, total flavonoid was 7-folds higher in intact plant compared to that of cell suspension culture of FDK1. Rutin production in intact plant was 4-folds higher compared to that of cell suspension culture of FDK1 while no naringin production was detected in cell suspension culture of FDK1 compared to 0.89 µg/g DW which was detected in intact plant. In conclusion, it is proven that *F. deltoidea* contains antioxidant activity and flavonoid contents.

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

**AKTIVITI ANTIOKSIDA DAN FLAVONOID DI DALAM PELBAGAI VARIETI
DAUN *FICUS DELTOIDEA* JACK (MAS COTEK)**

Oleh

MOHD HAKIMAN MANSOR

September 2013

Pengerusi: Professor Maziah Mahmood, PhD

Fakulti: Bioteknologi dan Sains Biomolekul

Ficus deltoidea Jack atau dikenali sebagai Mas Cotek di Malaysia adalah antara tumbuhan ubatan yang berpotensi untuk dikomersilkan kerana mempunyai aktiviti antioksidan yang tinggi. Sehingga kini, tiada maklumat mengenai varieti *F. deltoidea* yang terbaik dari segi aktiviti antioksidan untuk tujuan komersil. Oleh itu, kajian ini dijalankan untuk mengkaji aktiviti antioksidan dan kandungan flavonoid dalam tiga varieti *F. deltoidea* yang biasa digunakan sebagai herba ubatan di Malaysia. Objektif spesifik adalah untuk menentukan kapasiti antioksidan dalam ekstrak daun *F. deltoidea* menggunakan pelarut yang berbeza, menganalisis dan mengenal pasti kandungan flavonoid dalam ekstrak daun *F. deltoidea*, dan menyelidik pengeluaran flavonoid dalam ampai sel *F. deltoidea*. Dua pokok dari setiap varieti dipilih. Mereka adalah FDK1 dan FDK2 (var. *kunstleri*), FDT1 dan FDT2 (var. *trengganuensis*) dan FDA1 dan FDA2 (var. *angustifolia*). Daripada kajian ini, ekstrak daun *F. deltoidea* menunjukkan aktiviti antioksidan berbeza bergantung kepada kaedah dan pelarut yang digunakan. Ekstrak daun daripada var. *trengganuensis* mendapati kandungan protein dan gula penurun tertinggi adalah 30.6 dan 17.9 mg/g FW di dalam FDT2 dan FDT1, masing-masing manakala kandungan gula terlarut tertinggi adalah di dalam ekstrak daun FDA2 dengan 30.7 mg/g FW. Kandungan asid askorbik tertinggi didapati dalam ekstrak daun FDT1 dengan 4.12 mg/g FW. Aktiviti enzim antioksidan tertinggi di dalam ekstrak daun FDK1 untuk penentuan aktiviti katalase, peroksidase, askorbat peroksidase dan askorbat oksidase dengan 0.23, 0.75, 9.01 dan 23.20 unit/mg protein. Ekstrak akueus pada suhu bilik (25 °C) FDK1 menunjukkan aktiviti antioksidan tertinggi dengan

1.85 mg (bersamaan Trolox) TE/g berat segar (FW) menggunakan kaedah potensi penurunan ferik (FRAP) dan ekstrak daun FDT2 mengandungi jumlah kandungan antioksidan tertinggi menggunakan kaedah 1,1-diphenyl-2-picrylhydrazyl (DPPH) dengan 4.85 mg TE/g FW. Analisis antioksidan dan kandungan fenolik menggunakan ekstrak akueus daun *F. deltoidea* dijalankan secara panas dan sejuk dan mendapati ekstrak daun mengandungi antioksidan tinggi dalam ekstrak akueus panas berbanding dengan ekstrak akueus sejuk. Di samping itu, ekstrak metanol dan etanol *F. deltoidea* turut dianalisa dan didapati bahawa ekstrak etanol menghasilkan lebih banyak antioksidan berbanding dengan ekstrak metanol. Ekstrak daun *F. deltoidea* var. *kunstleri* (FDK1) mengandungi aktiviti polifenol tertinggi bagi ekstrak metanol dan etanol dengan 1.12 dan 1.13 mg GAE/g FW. Kandungan asid fenolik tertinggi ditemui dalam ekstrak daun FDK1 melalui ekstrak metanol dengan 4.54 mg GAE/g FW manakala melalui ekstrak etanol, ekstrak daun FDK2 menunjukkan aktiviti tertinggi dengan 4.00 mg GAE/g FW. Semua enam pokok mewakili tiga varieti dianalisa untuk kandungan flavonoid menggunakan kromatografi cecair berprestasi tinggi (HPLC). Eksperimen ini mendapati bahawa rutin dan naringin hadir dalam semua pokok di mana rutin lebih banyak daripada naringin. Ekstrak daun FDK1 menunjukkan kandungan rutin tertinggi dengan 12.83 µg/g berat kering (DW) manakala FDT2 mengandungi naringin tertinggi dengan 3.04 µg/g DW. Ampaian sel dibuat daripada kalus daun FDK1. Ampaian sel dimulai dengan membiakkan kalus di dalam medium cecair pada 120 rpm. Tahap keseragaman sel diperolehi dengan menyaring ampaian sel melalui penapis keluli tahan karat pada saiz yang berbeza. Ampaian sel FDK1 pada hari 12 dipilih untuk eksperimen seterusnya kerana menghasilkan flavonoid tertinggi dengan 2.10 mg RE/g DW. Saiz inokulum permulaan 2 g/kultur menghasilkan biojisim tertinggi dengan 0.65 g/25 mL media manakala penghasilan flavonoid tertinggi didapati dengan saiz inokulum permulaan 0.5 g/kultur dengan 3.3 mg RE/g DW. Biojisim dan penghasilan flavonoid ampaian sel kultur FDK1 berkadar terus dengan saiz sel agregat dengan 0.28 g/25 mL media dan 3.3 mg RE/g DW, masing-masing menggunakan penapis bersaiz 750 µm berbanding dengan saiz agregat yang lain. Nilai pH permulaan kontrol (pH 5.75) adalah pH terbaik untuk penghasilan biojisim dan flavonoid dengan 0.28 g/25 mL media dan 3.3 mg RE/g DW. Kandungan antioksidan dalam tumbuhan menggunakan kaedah DPPH mempunyai 3 kali ganda lebih tinggi berbanding ampaian sel FDK1 manakala 6 kali ganda menggunakan kaedah FRAP. Pada masa yang sama, kandungan flavonoid mengandungi 7 kali ganda lebih tinggi dalam tumbuhan berbanding ampaian sel FDK1. Penghasilan rutin dalam tumbuhan adalah 4 kali ganda lebih tinggi berbanding ampaian sel FDK1 manakala tiada penghasilan naringin didapati di dalam ampaian sel FDK1 berbanding 0.89 µg/g DW di dalam tumbuhan. Kesimpulannya, ampaian sel FDK1 boleh dijadikan kaedah pengkomersilan dengan kehadiran kandungan antioksidan dan flavonoid dalam kultur ampaian sel.

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“One can pay back the loan of gold, but one dies forever in debt to those who are kind” – Malay proverb.

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These thesis's dedicatees have more honour than a mere acknowledgements can bestow.

I certify that an Examination Committee has met on _____ to conduct the final examination of Mohd Hakim Mansor on his Doctor of Philosophy thesis entitled “Antioxidant and Flavonoid Activities in Different Varieties of *Ficus deltoidea* Jack (Mas Cotek) Leaves” in accordance with University Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulations 1981. The Committee recommends that the candidate be awarded the relevant degree. Members of the examination committee are as follows:

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