

ANTIOXIDATIVE ACTIVITIES AND ISOLATION OF
PHENOLIC COMPOUNDS FROM THE ROOT, FRUIT AND
LEAF OF MENSKUDU (*MORINDA CITRIFOLIA* L.)

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MASTER OF SCIENCE
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**ANTIOXIDATIVE ACTIVITIES AND ISOLATION OF PHENOLIC
COMPOUNDS FROM THE ROOT, FRUIT AND LEAF OF
MENGKUDU (*Morinda citrifolia* L.)**

By

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COMPOUNDS FROM THE ROOT, FRUIT AND LEAF OF MENCUNDE
(*Melicope chrysis* L.)

By

ZANZANARA BT MOHD ZIN

October 2011

Especially dedicated to.....

Chairman: Associate Professor Azman Abdul Razak, Ph.D

Faculty: Faculty of Science

Ayah and Ma

Along and Abang Wan

Kak Che, Mujahid, Kakak, Abg Mat, Kak Chik and Adik Faiz

Dina, Suhaila and Irfan

This study was conducted

Mohamad Khairi Mohd Zainol

extracts from different

and last but not least my princess

and root. Methanol, petroleum ether, chloroform and diethyl ether were used as extractive

Nur Fatin Aliya

thank you for your love, guidance, encouragement and doa's given throughout my life.....

(FTC) and superoxide

thank you for having faith in me.....

were then compared to that of α -tocopherol and butylated hydroxytoluene (BHT). Effect

of solvent types and concentration of extract used on antioxidative activities of *M.*

chrysis were also carried out. Meanwhile, isolation of phenolic compounds was carried

out using Sephadex LH-20 column chromatography. Identification and quantification of

the common flavonoids in the separated fractions were carried out using a reversed

phase high performance liquid chromatography (RP-HPLC) procedure on a Symmetry

C₁₈ column with water-miscible gradient elution system. Results showed that methanol

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COMPOUNDS FROM THE ROOT, FRUIT AND LEAF OF MENGKUDU
(*Morinda citrifolia* L.)**

By

ZAMZAHAILA BT MOHD ZIN

October 2003

Chairman : Associate Professor Azizah Abdul Hamid, Ph.D

Faculty : Food Science and Biotechnology

This study was conducted with the objective of evaluating the antioxidative activities of extracts from different parts of Mengkudu (*Morinda citrifolia* L.) including leaf, fruit and root. Methanol, petroleum ether, water and ethyl acetate were used as extraction solvent. The antioxidative activities assays were done using ferric thiocyanate method (FTC) and thiobarbituric acid test (TBA). Furthermore, the activities of these extracts were then compared to that of α -tocopherol and butylated hydroxyl toluene (BHT). Effect of solvent types and concentration of extract used on antioxidative activities of *M. citrifolia* were also carried out. Meanwhile, isolation of phenolic compounds was carried out using Sephadex LH-20 column chromatography. Identification and quantification of the common flavonoids in the separated fractions were carried out using a reversed phase high-performance liquid chromatography (RP-HPLC) procedures on a Symmetry C₁₈ column with water-methanol gradient elution system. Results showed that methanol root extract exhibited higher activity compared to that of fruit and leaf extract of the

same with no significant ($p < 0.05$) difference as compared to that of α -tocopherol and BHT. The result also showed that methanol gave highest yield of extract compared to that of water, petroleum ether and ethyl acetate extraction. Meanwhile, both polar and non-polar extract of *M. citrifolia* L. exhibited appreciable activity. Increasing concentration of methanol root extracts from 1000 to 5000 ppm do not significantly increased the antioxidative activity. On the other hand, there was significant ($p < 0.05$) difference in antioxidative activities of methanol leaf and fruit extracts when concentrations were increased. Similarly, increasing concentrations of water extracts from 1000 to 5000 ppm do not significantly increased the antioxidative activity of root and leaf of *M. citrifolia* L. However, there was significant ($p < 0.05$) difference in antioxidative activities of fruit extract at 1000 ppm compared to 2000 ppm. At 3000 ppm onwards there were no significant ($p < 0.05$) difference in activities. Result also showed that at 3000 ppm, water extract of root, fruit and leaf of *M. citrifolia* L. exhibited no significant ($p < 0.05$) difference in activities than that of either α -tocopherol or BHT. Meanwhile, increasing concentration of petroleum ether extract significantly increased the antioxidative activity of all parts of *M. citrifolia*. The result also revealed that the antioxidative activities exhibited by petroleum ether root extract was significantly ($p < 0.05$) higher than that of either α -tocopherol or BHT at concentration more than 4000 ppm. Similar observation was found in fruit and leaf extract of same solvent at 5000 ppm, where fruit and leaf extract exhibited significantly ($p < 0.05$) higher activity than that of either α -tocopherol or BHT. Increasing concentration of ethyl acetate leaf extract from 1000 ppm to 5000 ppm do not significantly increased the antioxidative activities. On the other hand, at 3000 ppm onwards there was significant ($p < 0.05$)

difference in antioxidative activities of root and fruit extracts when concentrations were increased. Result also showed that at 3000 ppm and 4000 ppm, there was no significant ($p < 0.05$) difference in the activities exhibited by root and leaf extract of same solvent than that of either α -tocopherol or BHT. Meanwhile ethyl acetate fruit extract exhibited significantly higher activity than that of either α -tocopherol or BHT at the same concentration tested. From this study it can be seen that different parts of *M. citrifolia* exhibited varying degrees of antioxidative activities when different solvents were used in extraction procedures. Of the four solvents used, ethyl acetate is the best solvent for extracting antioxidative compounds from different parts (root, fruit and leaf) of *Morinda citrifolia* L. compared to other solvent. Isolation of phenolic compounds mainly focused on the separation and identification of antioxidants from methanol extract because of the polar properties of phenolic compounds especially flavonoids, which are known to be potent antioxidant. All fractions were seen to contain different amount of total phenolic compound and exhibited considerably high antioxidative activity. Identification of flavonoid successfully determines the individual active compound present in *M. citrifolia*. Major component determine including catechin and epicatechin, which are known to be potent antioxidant.

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

**AKTIVITI ANTIOKSIDA DAN ISOLASI BAHAN FENOLIK DARIPADA
AKAR, BUAH DAN DAUN MENGGUDU (*Morinda citrifolia* L.)**

Oleh

ZAMZAHAILA BT MOHD ZIN

Oktober 2003

Pengerusi : Profesor Madya Dr. Azizah Abdul Hamid, Ph.D.

Fakulti : Sains Makanan dan Bioteknologi

Kajian ini dijalankan bertujuan untuk mengenalpasti kesan aktiviti antioksidasi oleh ekstrak daripada bahagian Mengkudu (*Morinda citrifolia* L.) yang berbeza termasuklah daun, buah dan akar. Metanol, petroleum eter, air dan etil acetate telah digunakan sebagai pelarut pengekstrakan. Ujian antioksidasi yang digunakan adalah ujian ferik thiosianat (FTC) dan ujian asid tiobarbiturik (TBA). Seterusnya, hasil daripada kajian ini dibandingkan dengan α -tokoferol and butylated hidroksil tolune (BHT). Selain itu, kesan jenis pelarut yang berlainan dan kesan kepekatan ekstrak yang digunakan juga dikaji. Disamping itu juga, Sephadex LH-20 telah digunakan dalam proses isolasi bahan fenolik dengan kaedah kromatografi turus. Seterusnya, pengenalpastian dan pengiraan beberapa jenis flavonoid yang biasa terdapat dalam tumbuhan dikenalpasti didalam fraksi-fraksi yang terhasil daripada proses kromatografi turus. Ini dilakukan dengan menggunakan kaedah kromatografi cecair bertekanan tinggi berfasa terbalik (RP-HPLC) berfasa pegun turus Symmetry C₁₈ dengan air dan methanol sebagai fasa gerak. Kajian ini mendapati, ekstrak akar dalam metanol menunjukkan kesan antioksidasi yang terbaik

manakala buah dan daun menunjukkan aktiviti yang lebih rendah yang juga tidak menunjukkan kesan yang ketara berbanding α -tocopherol dan BHT. Kajian ini juga mendapati, metanol merupakan pelarut yang paling baik dalam menghasilkan hasil ekstraksi yang terbanyak berbanding air, petroleum eter dan etil acetat. Selain itu, kedua-dua ekstrak polar dan tak polar Mengkudu menunjukkan kesan antioksidasi yang agak tinggi. Seterusnya, kajian ini juga mendapati peningkatan kepekatan ekstrak metanol bagi akar dari 1000 ppm ke 5000 ppm tidak meningkatkan aktiviti dengan ketara. Selain daripada itu, terdapat perbezaan yang ketara dalam aktiviti antioksidasi ekstrak metanol bagi daun dan buah apabila kepekatan bertambah. Kesan yang sama berlaku dalam ekstrak air bagi akar dan daun, dimana peningkatan kepekatan ekstrak tidak meningkatkan aktiviti antioksidasi dengan ketara. Tetapi, terdapat perbezaan yang ketara dalam aktiviti antioksidasi ekstrak buah pada kepekatan 1000 ppm berbanding 2000 ppm. Pada 3000 ppm keatas, tiada perbezaan yang ketara dalam aktiviti antioksidasi. Keputusan juga menunjukkan pada 3000 ppm, ekstrak air bagi akar, buah dan daun Mengkudu menunjukkan tiada perbezaan yang ketara dalam aktiviti dibandingkan dengan α -tocopherol dan BHT. Manakala, peningkatan kepekatan ekstrak petroleum eter, secara ketara meningkatkan aktiviti antioksidasi dalam semua bahagian Mengkudu. Keputusan juga mendapati aktiviti antioksidasi yang ditunjukkan oleh ekstrak petroleum eter bagi akar adalah lebih tinggi berbanding kedua-dua α -tocopherol dan BHT pada kepekatan 4000 ppm dan keatas. Pemerhatian yang sama didapati bagi ekstrak buah dan daun pada 5000 ppm, dimana kedua-duanya telah menunjukkan aktiviti yang lebih tinggi berbanding α -tocopherol dan BHT. Peningkatan kepekatan ekstrak etil acetate daun daripada 1000 ppm – 5000 ppm tidak meningkatkan aktiviti antioksidasi secara ketara.

Seterusnya, pada kepekatan 3000 ppm dan keatas, terdapat perbezaan yang ketara dalam aktiviti antioksidasi bagi akar dan buah bila kepekatan meningkat. Keputusan juga menunjukkan pada 3000 ppm dan 4000 ppm, tiada perbezaan yang ketara dalam aktiviti yang ditunjukkan oleh akar dan daun berbanding dengan kedua-dua α -tocopherol dan BHT. Disamping itu juga ekstrak etil acetate bagi buah menunjukkan aktiviti yang lebih tinggi berbanding kedua-dua α -tocopherol dan BHT pada kepekatan yang sama. Daripada kajian ini, didapati bahagian-bahagian yang berbeza pada Mengkudu menunjukkan darjah aktiviti antioksidasi yang berbeza bila pelarut yang berbeza digunakan dalam pengekstrakan. Daripada empat pelarut yang digunakan, etil acetate didapati merupakan pelarut yang paling baik untuk mengekstrak antioksidasi daripada pelbagai bahagian Mengkudu berbanding dengan pelarut lain. Pengisolasi bahan fenolik adalah ditumpukan kepada pemisahan dan pengenalpastian antioksidasi daripada ekstrak metanol disebabkan ciri polar yang terdapat pada komponen fenolik terutamanya flavonoid yang terkenal sebagai komponen aktif antioksidasi. Keseluruhan bahagian ekstrak yang dipisahkan didapati mempunyai jumlah komponen fenolik yang berbeza dan menunjukkan aktiviti antioksidasi yang tinggi. Pengenalpasti komponen-komponen aktif yang hadir dalam *M. citrifolia* mendapati komponen major yang hadir dalam *M. citrifolia* adalah catechin dan epicatechin, yang mana komponen ini telah dikenali sebagai komponen yang aktif antioksidasi.