UNIVERSITI PUTRA MALAYSIA

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

ZAMZAHAILA BT MOHD ZIN

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ZAMZAHAILA BT MOHD ZIN

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

October 2003

Especially dedicated to.....

Ayah and Ma
Along and Abang Wan
Kak Che, Mujahid, Kakak, Abg Mat, Kak Chik and Adik Faiz
Dina, Suhaila and Irfan
Mohamad Khairi Mohd Zainol
and last but not least my princess
Nur Fatin Aliya

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

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

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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 tolune (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 C_{18} 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 MENGKUDU (*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 antioksida 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 antioksida yang digunakan adalah ujian ferik thiosianat (FTC) dan ujian asid tiobarbiturik (TBA). Seterusnya, hasil daripada kajian ini dibandingkan dengan α-tokoferol and butilated 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 C_{18} dengan air dan methanol sebagai fasa gerak. Kajian ini mendapati, ekstrak akar dalam metanol menunjukkan kesan antioksida yang terbaik

manakala buah dan daun menujukkan 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 antioksida 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 antioksida 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 antioksida dengan ketara. Tetapi, terdapat perbezaan yang ketara dalam aktiviti antioksida ekstrak buah pada kepekatan 1000 ppm berbanding 2000 ppm. Pada 3000 ppm keatas, tiada perbezaan yang ketara dalam aktiviti antioksida. 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 antioksida dalam semua bahagian Mengkudu. Keputusan juga mendapati aktiviti antioksida 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. Penigkatan kepekatan ekstrak etil acetate daun daripada 1000 ppm – 5000 ppm tidak meningkatkan aktiviti antioksida secara ketara.

Seterusnya, pada kepekatan 3000 ppm dan keatas, terdapat perbezaan yang ketara dalam aktiviti antioksida bagi akar dan buah bila kepekatan meningkat. Keputusan juga menunjukkan pada 3000 ppm dan 4000 ppm, tiada perbezaan yang ketara dalam aktiviti yang ditujukkan 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 antioksida yang berbeza bila pelarut yang berbeza digunakan dalam pengekstrakan. Daripada empat pelarut yang digunakan, etil acetate didapati merupakan pelarut yang paling baik untuk mengekstrak antioksida daripada pelbagai bahagian Mengkudu berbanding dengan pelarut lain. Pengisolasi bahan fenolik adalah ditumpukan kepada pemisahan dan pengenalpastian antioksida daripada ekstrak metanol disebabkan ciri polar yang terdapat pada komponen fenolik terutamanya flavonoid yang terkenal sebagai komponen aktif antioksida.. Keseluruhan bahagian ekstrak yang dipisahkan didapati mempunyai jumlah komponen fenolik yang berbeza dan menunjukkan aktiviti antioksida 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 antioksida.

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I certify that an Examination Committee met on 5th September 2003to conduct the final examination of Zamzahaila Mohd Zin on her Master of Science thesis entitled "Antioxidative Activities and Isolation of Phenolic Compounds from the Root, Fruit and Leaf of Mengkudu (*Morinda citrifolia* L.)" in accordance with Universiti 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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This thesis submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirements for the degree of Master of Science. The members of the Supervisory Committee are as follows:

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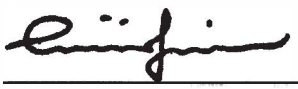

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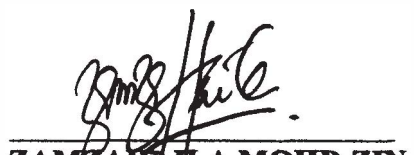
Date : 8 JAN 2004

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 degree at UPM or other institutions.



ZAMZAHAILA MOHD ZIN

Date: 31 OCT 2003

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LIST OF ABBREVIATIONS

BHA	Butylated hydroxyanisole
BHT	Butylated hydroxytoluene
DPPH	2,2-diphenyl-1-picrylhydrazyl
FTC	Ferric thiocyanate
h	Hour
HCl	Hydrochloric acid
HPLC	Reverse phase high pressure liquid chromatography
L	Liter
LDL	Low density lipoprotein
mg	Milligram
ml	Milliliter
nm	Nanometer
mm	Millimeter
µg	Microgram
µm	Micrometer
mM	MilliMolar
MAD	Malonaldehyde
MeOH	Methanol
Min	Minutes
N	Normality
Na DEDTC	Sodium diethyldithiocarbamic
PG	Propyl gallate
RP-HPLC	Reverse phase high pressure liquid chromatography
ppm	Part per million
rpm	Revolution per minute
SAS	Statistical Analysis System
TFA	Trifluoroacetic acid
TBA	Thiobarbituric acid
UV	Ultraviolet
UV/Vis	Ultraviolet / visible
v/v	Volume per volume.
w/v	Weight per volume

CHAPTER 1

GENERAL INTRODUCTION

The tale of the Hawaiian noni (*Morinda citrifolia*) plant likely began 1500 years ago with the arrival of the early Polynesian settlers to Hawaii (Abbott and Shimazu, 1985). Archaeological evidence suggests that the first settlers were from the Marquesas Islands (Feher, 1969; Mitchell, 1992), and in fact the Marquesans and Hawaiian use the same name for the noni, compared to Samoans and Tongans (nonu) or Rarotongans (nono) (Uhe, 1974). The ancient Polynesians likely brought along the noni for its use as a food, dye and medicine (Abbott and Shimazu, 1985). They consumed the fruits during times of famine (Degener, 1973), coloured their kapa (Hawaiian cloth) yellow and red with roots (Mitchell, 1992) and treated numerous ailments with various portions of the plant (Chun, 1994).

Morinda citrifolia must have easily taken root in the new land because of the warm tropical climate. Even to this day, it can be found growing near abandoned ancient dwellings (Degener, 1973), scattered along rocky lava coastlines and cultivated in lowland gardens (Merlin, 1977). Although the historical accounts are few, the most intriguing aspect of the *M. citrifolia* is its professed healing power. It was highly valued for its medicinal properties (Kepler, 1983), in which virtually the whole plant (fruit, leaf, bark, root, flower and seed) had been used.

In the Tropics it seems to have much valued medicinally, the genus is cultivated for its roots, also at times for leaves and fruits. Most part of the tree has been widely used medicinally since ancient times. The plant portions treated numerous

conditions, including some that were infectious in origin. Combination of the bark, fruit and other plant products was antidotal against tuberculosis. The flower or fruit with additional plant material treated thrush, a fungal infection (Chun, 1994). The fruit also acted as a poultice to "draw out the pus and core from an infected sore or boil" (Krauss, 1981) and alleviated "itching of the anus due to pinworms" (Chun, 1994). Other sundry remedies included purifying the blood and stimulating the appetite with a root concoction (Chun, 1994), healing broken bones, "especially if there was a compound fracture" with the unripe fruit (Krauss, 1981), treating rheumatic joints (Gutmanis, 1994) or "sprains of knuckles or wrists and of the knees" (Chun, 1994) with the leaf, helping kidney and bladder problems with the flower and fruit (Gutmanis, 1994) and medicating heart troubles with the juice of the ripe fruit (McBride, 1975).

The traditional healing applications of *M. citrifolia* in Hawaii are comparable with other regions of the Pacific. This might be expected since the early Polynesian immigrants would have brought with them their customs and knowledge of plants from their former homeland. In general, the Polynesians mainly used the fruits or leaves of the plants to treat infectious and inflammatory disorders, such as boils, oral infections and rheumatism. Sometimes the natives from one Pacific Island utilised a different portion of plant to treat an analogous ailment. For example, in Hawaii the noni fruit with other agents treated 'gripping pain of the stomach or intestines" (Chun, 1994), whereas in Tonga it was the bark or leaves that medicated stomach-aches (Whistler, 1992). Female gynecological problems such as pain after childbirth were treated with the flowers in Ponape (Glassman, 1952), while the leaves were used to alleviate pregnancy pain in Fiji (Cambie and Ash, 1994). The application of

the various plant sections to treat similar conditions may indicate the existence of a common active component throughout the plant. Also, it is interesting to note that the plant occasionally treated opposing ailment. The fruit was useful for constipation in Hawaii (Chun, 1994), and for diarrhoea in Gilbert Island (Cambie and Ash, 1994). The bark and roots were used as homeostatic for menstruation in Penope (Glassman, 1952) while the bark or leaves treated amenorrhea in Tonga (Singh *et al.*, 1984).

Other regions of the world also recorded folkloric uses of the *M. citrifolia* plant. The *M. citrifolia* plants can be found in parts of tropical Africa, America, Asia and Australia, although it may be originally native only to the island of Indonesia (Whistler, 1992). In parts of Central America, it was commonly referred to as "painkiller" because of the leaves, which were useful in relieving pain (Little and Wadsworth, 1964). In Barbados, the fruit was believe to be poisonous and was called "Forbidden Fruit" (Gooding *et al.*, 1965). This is in contrast to Burma, where the fruits were considered edible and were consumed raw with salt or cooked in curries (Morton, 1992). In other neighbouring areas such as Vietnam, Laos and Cambodia, the fruits, leaves and roots were used as medicine (Perry and Metzger, 1980). For the most part, the *M. citrifolia* had similar medicinal application around the world. The plants was commonly used for fevers, sore throats, cough, stomach-aches, ulcers, diarrhea, dysentery, pain, boils, wounds, arthritis and worms. Less common was its used for asthma, tuberculosis, hypertension, diabetes, malaria, liver ailments and splenomegaly. The universal recognition of *M. citrifolia* as a traditional healing remedy establishes a sense of credibility for the medicinal lore of this plant.