



UNIVERSITI PUTRA MALAYSIA

***ANTIMICROBIAL ACTIVITY OF MELASTOMA MALABATHRICUM LINN.
FLOWER AND FRUIT CRUDE EXTRACTS***

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FLOWER AND FRUIT CRUDE EXTRACTS**

By

SITI NURHADIS BINTI CHE OMAR

**Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in
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June 2012

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

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Chairman: Janna Ong Abdullah, PhD

Faculty: Biotechnology and Biomolecular Sciences

Natural products are rich sources of antimicrobial compounds with broad spectrum and sufficiently good pharmacokinetics to be clinically useful without chemical modifications. With the increase in microbial resistance to antibiotics, there is considerable interest in investigating the antimicrobial effects of potential plant extracts as potential sources for developing natural antimicrobial agents. *Melastoma malabathricum* Linn. is a shrub that belongs to the family Melastomataceae and a common herbal plant used in folk medicines to treat inflamed wounds and other ailments. It is commonly found in Malaysia with beautiful pink or purple flowers and berries-like fruits rich in anthocyanins. Hence, this study was carried out with the aim to evaluate the inhibitory activities of different concentrations of the *M. malabathricum* Linn. flower and fruit crude extracts against a variety of microorganisms, which comprised of 12 Gram-positive bacteria, 17 Gram-negative bacteria and three fungi species using the disc diffusion method. The lowest concentrations of the extracts

producing inhibition zones against the test microorganisms were used to determine their Minimum Inhibitory Concentrations (MICs) and Minimum Bactericidal Concentrations (MBCs) or Minimum Fungicidal Concentrations (MFCs). In addition, the effects of different temperatures (4°C, 25 °C and 37 °C) and pHs (4, 6, 7 and 8) on the stability of the crude extracts correlating to their growth inhibitory ability were determined for *Listeria monocytogenes* IMR L55 and *Staphylococcus aureus* IMR S244 grown in medium supplemented with the respective crude extracts at different temperatures and pHs. *In vitro* cytotoxicity effect of the *M. malabathricum* Linn. extracts on the human Chang liver and 3T3- Fibroblast cells using the MTT assay was also carried out. Overall, the Gram-positive bacteria were more susceptible to both crude extracts compared to the Gram-negative species. The results showed that both crude flower and fruit extracts exhibited strong inhibitory activities against *Micrococcus luteus* but had no effects on all fungi tested. The MIC values for the crude flower and fruit extracts on all the bacteria tested ranged from 12.5 to 100.0 mg/ml. While, the average MBC values were from 50.0 to 100.0 mg/ml for both extracts. *L. monocytogenes* IMR L55 and *S. aureus* IMR S244 exhibited the highest sensitivity to both crude extracts at 100 mg/ml. The crude flower extract was more effective in inhibiting the growth of *L. monocytogenes* IMR L55 in which a reduction of 4.5 to 8.0 log CFU/ml was detected when tested at pHs 4, 6, and 7 for 4 °C, 25 °C and 37 °C. While, the crude fruit extract was more effective in reducing the growth of *S.aureus* IMR S244 with 4.5 to 8.0 log CFU/ml reduction at pHs 4, 6, 7 and 8 for 25 °C and 37 °C. The MTT assay showed that the crude fruit extract exhibited an IC₅₀ of 0.70 mg/ml when tested on Chang liver cells after 48 hours of treatment compared to the crude flower extract (IC₅₀ ≥ 1.0 mg/ml); whereas the 3T3-

Fibroblast cells incubated with the flower and fruit extracts showed an IC_{50} of 0.40 mg/ml and 0.60 mg/ml, respectively. Overall, the results obtained in this study pinpoint that both extracts have potentials to inhibit or kill selective bacterial pathogens, with the flower extract exhibiting better antibacterial activity compared to the fruit extract. The data also suggests the possible uses of the extracts to control selective pathogens and as natural sources for the discovery of natural antibacterial agent(s) in the future.



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

**AKTIVITI ANTIBAKTERIA PADA EKSTRAK BUNGA DAN BUAH
MELASTOMA MALABATHRICUM LINN.**

oleh

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Produk semulajadi adalah sumber yang kaya dengan sebatian antimikrobial dengan spektrum yang luas dan farmakokinetik yang baik untuk kegunaan klinikal tanpa modifikasi kimia. Dengan peningkatan rintangan mikroorganisma terhadap antibiotik, tumpuan kepada penyelidikan untuk mencari agen semulajadi antimikrob daripada ekstrak-ekstrak tumbuhan kian meningkat. *Melastoma malabathricum* L. merupakan tumbuhan yang berasal dari keluarga Melastomataceae dan ia adalah pokok herba yang biasa digunakan dalam perubatan tradisional untuk merawat luka dan penyakit lain. Pokok ini mudah dijumpai di Malaysia dan mempunyai bunga berwarna merah jambu atau ungu dan buah yang kaya dengan antosianin. Oleh demikian, kajian ini dijalankan bertujuan untuk menilai aktiviti perencatan pada pertumbuhan 12 bakteria Gram-positif, 17 bakteria Gram-negatif dan tiga spesis kulat oleh ekstrak bunga dan buah tumbuhan ini pada kepekatan yang berlainan dengan menggunakan kaedah penyebaran disk. Zon perencatan yang dihasilkan oleh mikroorganisma yang telah diuji pada kepekatan ekstrak

yang terendah telah digunakan untuk menentukan nilai kepekatan minima perencatan (MICs) dan nilai kepekatan minima kebolehan membunuh bakteria (MBCs) dan kepekatan minima kebolehan membunuh kulat (MFCs). Di samping itu, kesan suhu (4° C, 25° C dan 37° C) dan pH (4, 6, 7 dan 8) pada kestabilan ekstrak juga diuji untuk melihat potensi ekstrak tersebut merencat pertumbuhan *Listeria monocytogenes* IMR L55 and *Staphylococcus aureus* IMR S244. Kajian ini juga menguji kesan ketoksikan ekstrak tumbuhan in pada pertumbuhan sel hati manusia ('human Chang liver cell') dan kulit ('3T3-Fibroblast cell') menggunakan kaedah MTT. Pada keseluruhannya, kajian ini menunjukkan bakteria Gram positif adalah lebih sensitif berbanding bakteria Gram negative terhadap kedua-dua ekstrak. Kedua-dua ekstrak menunjukkan kesan perencatan yang tinggi pada *Micrococcus luteus* dan tiada kesan perencatan pada kulat. Nilai MIC untuk ekstrak bunga dan buah pada semua mikroorganisma yang diuji adalah di antara 12.5 hingga 100.0 mg/ml. Manakala, nilai purata bagi MBCs adalah di antara 50.0 hingga 100.0 mg/ml. Pada kajian ke atas kesan suhu dan pH, kedua-dua ekstrak telah merencatkan pertumbuhan *Listeria monocytogenes* IMR L55 and *Staphylococcus aureus* IMR S244 pada 100 mg/ml kepekatan ekstrak yang diuji. Kesan perencatan menunjukkan perbezaan ketara pada ekstrak bunga berbanding dengan ekstrak buah apabila pertumbuhan *Listeria monocytogenes* IMR L55 diperolehi pada pH 4, 6, dan 7 untuk semua suhu yang diuji (4°C, 25 °C dan 37 °C) kekurangan pertumbuhan kira-kira 4.5 ke 8.0-log CFU/ml. Sedangkan pertumbuhan *Staphylococcus aureus* IMR S244 pada suhu 25 °C dan 37 °C telah menunjukkan kesan perencatan yang ketara oleh ekstrak buah berbanding ekstrak bunga pada pH 4, 6, 7 dan 8 dengan kesan kekurangan pertumbuhan kira-kira 4.0 ke 8.0-log CFU/ml. Nilai IC₅₀ bagi ekstrak buah yang diuji pada sel hati

melalui ujian MTT adalah 0.75 mg/ml berbanding ≥ 1.0 mg/ml bagi ekstrak bunga selepas 48 jam diuji. Manakala, untuk fibroblast pula, nilai IC_{50} bagi kedua-dua ekstrak adalah 0.40 mg/ml dan 0.60 mg/ml selepas 48 jam diuji. Pada keseluruhan, hasil kajian ini menunjukkan bahawa kedua-dua ekstrak mempunyai potensi berkemungkinan diguna untuk merencat ataupun membunuh pembiakan sesetengah bakteria, dan ekstrak bunga menunjukkan aktiviti antibakteria yang lebih baik berbanding ekstrak buah. Data yang diperolehi mencadangkan ekstrak ini boleh digunapakai untuk mengawal pembiakan atau pertumbuhan bakteria patogenik dan juga sebagai sumber semulajadi agen antibakteria yang baru pada masa akan datang.

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APPROVAL

I certify that a Thesis Examination Committee has met on 28 June 2012 to conduct the final examination of Siti Nurhadis Binti Che Omar on her thesis entitled "Antimicrobial Activity of *Melastoma malabathricum* Linn. Flower and Fruit Crude Extracts" 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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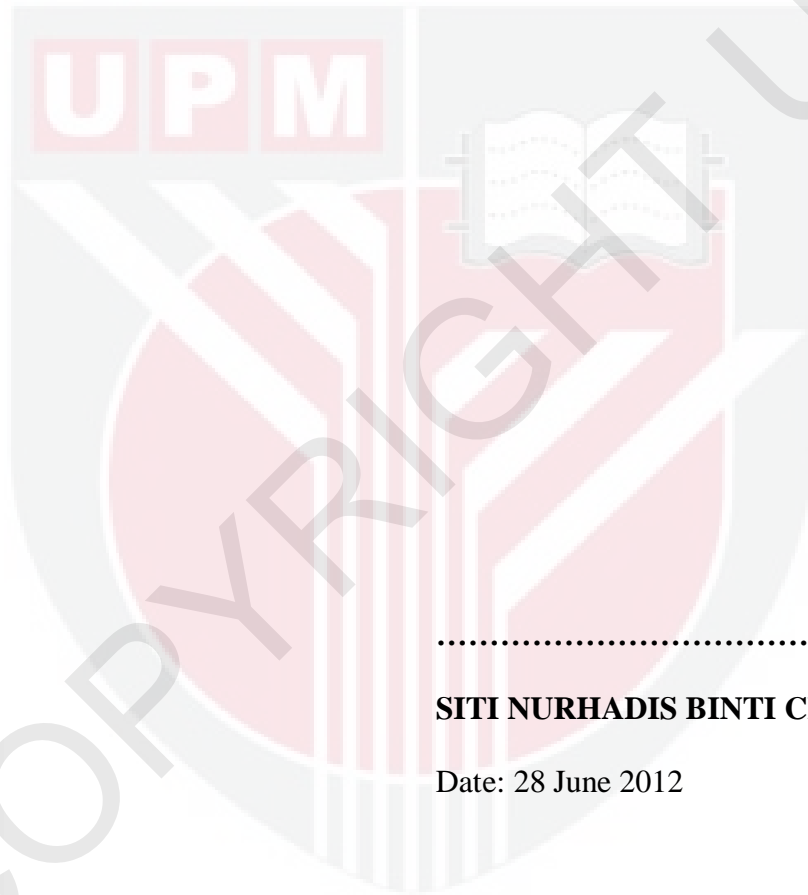
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DECLARATION

I declare 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 concurrently, submitted for any other degree at Universiti Putra Malaysia or at any other institution.



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SITI NURHADIS BINTI CHE OMAR

Date: 28 June 2012

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LIST OF ABBREVIATIONS

FAO	-	Food and Agriculture Organization
h	-	hour
g	-	gram
mg	-	milligram
mg/ml	-	milligram per milliliter
ml	-	milliliter
MBC	-	Minimum Bactericidal Concentration
MIC	-	Minimum Inhibitory Concentration
MMC	-	Minimum Microbicidal Concentration
MTT	-	(3-(4,5-dimethylthiazol-2-yl)-2,5- diphenyl tetrazolium bromide)
NA	-	Nutrient Agar
PBS	-	Phosphate buffer saline
PDA	-	Potato Dextrose Agar

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