# PHYTOCHEMICAL STUDIES AND BIOACTIVITY OF SELECTED SPECIES FROM THE FAMILY MELASTOMATACEAE

**NORFAIZAH MAHMUD**

**FACULTY OF SCIENCE**
**UNIVERSITY OF MALAYA**
**KUALA LUMPUR**

**2014**

# PHYTOCHEMICAL STUDIES AND BIOACTIVITY OF SELECTED SPECIES FROM THE FAMILY MELASTOMATACEAE

**NORFAIZAH BINTI MAHMUD**

**DISSERTATION SUBMITTED IN FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF BIOTECHNOLOGY**

**INSTITUTE OF BIOLOGICAL SCIENCES**
**FACULTY OF SCIENCE**
**UNIVERSITY OF MALAYA**
**KUALA LUMPUR**

**2014**

# UNIVERSITY OF MALAYA

## ORIGINAL OF LITERARY WORK DECLARATION

Name of Candidate : **Norfaizah Mahmud**

I/C/Passport No. : **890422-05-5130**

Registration / Matric No. : **SGF 120005**

Name of Degree : **Master of Biotechnology**

Title of Dissertation :

**"PHYTOCHEMICAL STUDIES AND BIOACTIVITY OF SELECTED SPECIES FROM THE FAMILY MELASTOMATACEAE"**

Field of Study : **Bioactivity of Natural Product**

I do solemnly and sincerely declare that:

(1) I am the sole author/writer of this Work;
(2) This Work is original;
(3) Any use of my work in which copyright exists was done by way of fair dealing and for permitted purposes and any excerpt or extract from, or reference to or reproduction of any copyright work has been disclosed expressly and sufficiently and the title of the Work and its authorship have been acknowledge in this Work;
(4) I do not have any actual knowledge nor do I ought reasonably to know that the making of this work constitutes an infringement of any copyright work;
(5) I hereby assign all and every rights in the copyright to this Work to the University of Malaya ("UM"), who henceforth shall be owner of the copyright in this Work and that any reproduction or use in any form or by any means whatsoever is prohibited without the written consent of UM having been first had and obtained;
(6) I am fully aware that if in the course of making this Work I have infringed any copyright whether intentionally or otherwise, I may be subjected to legal action or any other action as may be determined by UM.

Candidate's Signature Date :

Subscribed and solemnly declared before,

Witness's Signature Date :

Name:
Designation:

Witness's Signature Date :

Name:
Designation:

## ABSTRACT

The Melastomataceae is a large family of flowering plants consisting of trees, shrubs as well as epiphytes and are found throughout the tropics including Malaysia. However, only a handful of species have been studied for their phytochemical properties including the ones found in Malaysia. The present study was undertaken to evaluate the cytotoxic activities of the crude methanol (CME), ethyl acetate (CEE) and hexane extracts (CHE) of four selected wild species namely *Melastoma muticum, Melastoma sanguineum, Memecylon caeruleum* and *Phyllagathis rotundifolia*. Extracts of these species were prepared from the leaves and cytotoxic activities were screened by an *in vitro* assay system of growth inhibition against human breast MCF-7 cancer cell lines and human ovarian SKOV-3 cancer cell lines. CEE of *P. rotundifolia* exhibited the most potent cytotoxicity on MCF-7 cell lines ($IC_{50}$ = 0.92 ± 0.01 µg/ml, SI = 8.22), while CEE of *M. caeruleum* possessed high cytotoxicity on SKOV-3 cell lines ($IC_{50}$ = 16.04 ± 1.68 µg/ml, SI = 3.26). Qualitative phytochemical studies of the CME of the selected species revealed the presence of phenols, flavonoids, and tannins. Identification of the major compounds in CEE of *P. rotundifolia* and *M. caeruleum* were done by using LC-MS/MS system and phenolic groups were detected as the major compounds in both extracts. Total Phenolic Content (TPC) assay was done to measure the phenolic contents of each extract and CME of *M. sanguineum* exhibited the highest phenolic contents (718.18 ± 8.91 mg GAE/100 g). Antioxidant properties were studied by Ferric Reducing Antioxidant Power (FRAP) and 2,2-diphenyl-1-picrylhydrazyl (DPPH) assays and CME of *M. sanguineum* was found to show the highest reducing activities (68.89 ± 0.50 µM/100 g) while CME of *M. muticum* exhibited the high free radical scavenging activities ($IC_{50}$ = 77.1 ± 2.8 µg/ml).

## ABSTRAK

Melastomataceae ialah famili tumbuhan berbunga yang besar terdiri daripada pepohon, tumbuhan renek, dan juga epifit dijumpai di kawasan tropik termasuk Malaysia. Walau bagaimanapun, hanya segelintir spesis sahaja yang dikaji untuk kandungan serta aktiviti fitokimia, termasuk spesis yang dijumpai di Malaysia. Kajian ini dijalankan untuk mengkaji aktiviti sitotoksik ekstrak metanol (CME), etil asetat (CEE), and heksana (CHE) daripada empat spesis terdiri daripada *Melastoma muticum, Melastoma sanguineum, Memecylon caeruleum,* dan *Phyllagathis rotundifolia*. Ekstrak daun daripada spesis disediakan dan aktiviti sitotoksik di uji menggunakan assai '*in vitro'* dengan sistem halangan pertumbuhan sel MCF-7 kanser payudara manusia dan sel SKOV-3 kanser ovari manusia. CEE daripada *P. rotundifolia* menunjukkan aktiviti sitotoksik paling tinggi terhadap sel MCF-7 ($IC_{50}$ = 0.92 ± 0.01 µg/ml, SI = 8.22) manakala, CEE daripada *M. caeruleum* pula mempunyai aktiviti sitotoksik terhadap sel SKOV-3 ($IC_{50}$ = 16.04 ± 1.68 µg/ml, SI = 3.26). Kajian fitokimia secara kualitatif ke atas CME spesis terpilih menunjukkan kehadiran fenol, flavonoid, dan tanin. Pengenalpastian sebatian utama di dalam CEE *P. rotundifolia* dan *M. caeruleum* dilakukan menggunakan sistem LC-MS/MS dan didapati kumpulan fenolik adalah bahan utama yang dikesan di dalam kedua-dua ekstrak. Kandungan fenolik setiap ekstrak di ukur dengan menggunakan assai '*Total Phenolic Content*' (TPC) dan CME daripada *M. sanguineum* menunjukkan kandungan fenolik tertinggi (718.18 ± 8.91 mg GAE/100 g). Potensi antioksidan menggunakan '*Ferric Reducing Antioxidant Power*' (FRAP) dan '*2,2-diphenyl-1-picrylhydrazyl*' (DPPH) telah dikaji dan ekstrak CME daripada *M. sanguineum* menunjukkan aktiviti pengurangan oksida tertinggi (68.89 ± 0.50 µM/100 g) sementara itu CME daripada *M. muticum* pula mempamerkan aktiviti pengaut radikal bebas tinggi ($IC_{50}$ = 77.1 ± 2.8 µg/ml).

# ACKNOWLEDGEMENTS

First and foremost, Alhamdulillah (all praise to Allah). I cannot accomplish my study without His permission. Next, I would like to acknowledge my gratitude to my beloved supervisors, Dr. Sujatha Ramasamy and Dr. Sugumaran Manickam from the Institute of Biological Sciences. Dr. Sujatha had assisted with all the tissue culture works and Dr. Sugumaran had guided me in the taxonomical studies of the selected species in Melastomataceae. They have given excellent guidance based on their experiences and knowledge and provided constant support from beginning till the end of this study.

Special thanks to En. Roslan and all the lab technicians of the Biohealth Science Lab for their invaluable assistance and support. Without them, all my lab work would have not been completed on time. I would like to specially extend my utmost gratitude to Cik Siti Rogayah from the Biohealth Science Lab, for her consistent help and encouragement given throughout this study. I was so touched to find out that she rushed from her work to attend my Candidature Defence presentation. I take this as the most valuable moral support ever given to me for this work which I will never forget and will forever treasure this in my heart.

To all the lecturers involved in the M. Biotech coursework 2012/2013, thank you for your patience in teaching and sharing your precious experiences. I have gained a lot of knowledge as well as invaluable new experiences. To all my friends from the M. Biotech class 2012/2013, thank you for making the one-coursework-semester "alive and happening". The class would be so mundane without all of you. Also, I am so grateful that we have helped each other throughout the coursework. Thank you for all the feedback or comments and also thank you for providing assistance especially when I encountered problems in doing my research.

To my friends, especially Mimi, Fadhilah and Fatimah, thank you for the non-stop support, encouragements and contributions towards this project. I am ever so grateful to have friends that always stay by my side and encourage me to complete this study. Likewise, I am praying that all their current research will proceed smoothly and be completed soon.

Last but not least, I would like to express my uttermost appreciation to my beloved family members – Mama, Ayah, Kakyong & Abang Asrul, Kakngah & Abang Razak, Kak Lin and Adek. I might not be able to be where I am right now without their continuous support and prayers. Special thanks to Mama and Ayah, for trusting my decisions, for not judging or blaming me on any failures and for enjoying successful moments together. I don't really know how to describe all the feelings in words. But, I just feel blessed and thankful for having such a wonderful family.

**Norfaizah**
University of Malaya, 2014

# TABLE OF CONTENTS


| Content | Page |
|---|---|
| ABSTRACT | iii |
| ABSTRAK | iv |
| ACKNOWLEDGEMENTS | v |
| LIST OF FIGURES | x |
| LIST OF TABLES | xii |
| LIST OF SYMBOLS AND ABBREVIATIONS | xiv |
| LIST OF APPENDICES | xvi |
| **CHAPTER 1. INTRODUCTION** | 1 |
| **CHAPTER 2. LITERATURE REVIEW** | |
| 2.1 Plants and Medicinal Properties | 4 |
| 2.2 Therapeutic Values of the Species in the Genus *Melastoma* | 6 |
| 2.2.1 Brief Description of the Species in the Genus *Melastoma* used in this Study (*Melastoma muticum* and *Melastoma sanguineum*) | 10 |
| 2.3 Brief Description and Traditional Folklore uses of *Phyllagathis rotundifolia* | 11 |
| 2.4 The Genus *Memecylon* and Associated Species with Therapeutic Records | 12 |
| 2.4.1 A Short Description of *Memecylon caeruleum* | 14 |
| 2.5 Plant Natural Products | 15 |
| 2.5.1 The Discovery and Development of Natural Products as Anticancer Agents | 16 |
| 2.5.1.1 Alkaloids as Therapeutic Drugs and Pharmaceutical Agents | 18 |
| 2.5.1.2 Terpenoids as Therapeutic Drugs and Pharmaceutical Agents | 21 |
| 2.5.1.2.1 Saponins as Therapeutic Drugs and Pharmaceutical Agents | 23 |
| 2.5.1.3 Phenolic Compounds as Therapeutic Drugs and Pharmaceutical Agents | 25 |
| 2.5.1.3.1 Flavonoids as Therapeutic Drugs and Pharmaceutical Agents | 26 |
| 2.6 Cancer – A Brief Overview | 27 |
| 2.6.1 Brief Introduction to Breast Cancer | 29 |
| 2.6.2 Ovarian Cancer – Women's Silent Killer | 30 |
| 2.6.3 Cell Cycle – Phases of Cells Development and Proliferation | 31 |

2.7 Overview of Antioxidants 33
2.7.1 Free Radicals in Relation to Cancer 35
2.7.2 Antioxidants as Anticancer Agents 37

**CHAPTER 3. METHODOLOGY**

3.1 Macroscopic Studies 41

3.2 Preparation of Crude Extracts 41
3.2.1 Solvents 41
3.2.2 Sample Collection 42
3.2.3 Extraction of Plant Materials 42

3.3 Phytochemical Screening 45

3.4 Cytotoxicity Screening of Crude Extracts 48
3.4.1 Chemicals 48
3.4.2 Cell Lines 48
3.4.3 Cytotoxic Assay – Neutral Red Uptakes (NRU) Cytotoxic Assay 49

3.5 LC-MS/MS Analysis 51

3.6 Antioxidant Assay 52
3.6.1 Chemicals and Reagents 52
3.6.2 2,2-diphenyl-1-picrylhydrazyl (DPPH) Radical Scavenging Activity Assay 52
3.6.3 Ferric Reducing Antioxidant Power (FRAP) Assay 53
3.6.4 Determination of Total Phenolic Content (TPC) 53

3.7 Data Analysis 54

**CHAPTER 4. RESULTS**

4.1 Collection of the Selected Species in Melastomataceae 55

4.2 Macroscopic Characteristics of the Plants 56
4.2.1 *Memecylon caeruleum* Jack 56
4.2.2 *Melastoma muticum* Ridl. 57
4.2.3 *Melastoma sanguineum* Sims 58
4.2.4 *Phyllagathis rotundifolia* Bl. 59

4.3 Yield of Crude Extracts 60

4.4 Phytochemical Screening 60

4.5 Cytotoxic Activity of Crude Extracts on Human Cancer Cell Lines 62

4.6 Identification of Major Compound by using LC-MS/MS System in Crude Ethyl Acetate Extracts (CEE) of *M. caeruleum* and *P. rotundifolia* 66

4.7 Antioxidant Potential of Crude Extracts 70
4.7.1 2,2-diphenyl-1-picrylhydrazyl (DPPH) Radical Scavenging Activity Assay 70
4.7.2 Ferric Reducing Antioxidant Power (FRAP) Assay 71
4.7.3 Total Phenolic Content (TPC) Assay 72
4.7.4 Comparison of Antioxidant Activities 73
4.7.5 Correlation between Total Phenolic Content and Antioxidant Properties 74

**CHAPTER 5. DISCUSSION**

5.1 Botanical Identity and Pharmacognostical Study 76

5.2 Preparation of Crude Extracts 77

5.3 Preliminary Phytochemical Screening 79

5.4 Cytotoxic Screening of Crude Extracts on Various Human Cancer Cell Lines 81

5.5 LC-MS/MS Analysis of Cytotoxically Active Crude Extracts 84

5.6 Antioxidant Evaluation of Crude Extracts 87

**CHAPTER 6. CONCLUSION** 92

**REFERENCES** 93

**APPENDICES** 112

# LIST OF FIGURES


| Figure | | Page |
|---|---|---|
| 2.1(a) | Shrubby habit of *M. muticum* | 11 |
| 2.1(b) | Flowers of *M. muticum* with violet petals | 11 |
| 2.2(a) | Shrubby habit of *M. sanguineum* | 11 |
| 2.2(b) | Leaves of *M. sanguineum* | 11 |
| 2.3 | *P. rotundifolia* is a small plant usually found growing as forest floor herbs | 12 |
| 2.4(a) | Part of the flower of *M. caeruleum* with an attractive bluish base | 14 |
| 2.4(b) | Pink to dark red fruits of *M. caeruleum* | 14 |
| 2.5 | Structure of morphine, containing Nitrogen atom and ring structure | 18 |
| 2.6 | The chemical structure of berberine, evodiamine, matrine, piperine, sanguinerine, and tetrandrine | 20 |
| 2.7 | Structures of representative volatile terpenoid compounds | 22 |
| 2.8 | Core structure of triterpenoid sapogenins with arrangement of ring ABCDE | 24 |
| 2.9 | Basic structure of phenolic compounds (A = cinnamic acid; B = benzoic acid) | 25 |
| 2.10 | General structure of flavonoids | 26 |
| 2.11 | Process of metastasis. Cells grow as a benign tumour in epithelium in which they breakthrough the basal lamina and invade the capillary. The tumour cells travel through bloodstream (less than 1 in 1000 cells will survive to form metastasizes). The survived cells adhere to blood vessel wall in liver. Then, they escape from the blood vessel and proliferate to form metastasis in liver | 28 |
| 2.12 | Phases of cell cycle | 32 |
| 2.13 | Classification of dietary antioxidant. This classification excludes components of enzymatic antioxidants (eg: selenium and molybdenum) and dietary factors (eg: fatty acids) that may decrease oxidative stress via anti-inflammatory effects | 35 |

| | | |
|---|---|---|
| 2.14 | Three-stage model of carcinogenesis and the level of carcinogenic effects | 36 |
| 2.15 | Free radicals can act dual roles as cancer inhibitors or accelerators under different condition. COX-2: cyclooxygenase 2; IL-β: interleukin beta; INF-γ: interferon-gamma; p21: tumour protein 21; p53: tumour protein 53; RNS: reactive nitrogen species; ROS: reactive oxygen species; TNF-α: tumour necrosis factor alpha | 37 |
| 3.1 | Schematic extraction of the selected species from the family Melastomataceae | 44 |
| 4.1 | *Memecylon caeruleum* Jack | 56 |
| 4.2 | *Melastoma sanguineum* Sims | 57 |
| 4.3 | *Melastoma muticum* Ridl. | 58 |
| 4.4 | *Phyllagathis rotundifolia* Bl. | 59 |
| 4.5 | Dose-response curve of CME of *M. muticum* tested against human ovarian SKOV-3 cancer cell line | 62 |
| 4.3 | LC-MS/MS profiles of chemical compounds in CEE of *M. caeruleum* | 68 |
| 4.4 | LC-MS/MS profiles of chemical compounds in CEE of *P. rotundifolia* | 69 |

# LIST OF TABLES


| Table | | Page |
|---|---|---|
| 2.1 | Reported medicinal uses of *M. malabathricum* by various communities/tribes around the world | 8 |
| 2.2 | Scientific findings related to pharmacological properties of selected *Melastoma* species | 9 |
| 2.3 | Medicinal properties of selected *Memecylon* species | 13 |
| 2.4 | Plant-derived anticancer agents used in preclinical and clinical stages | 17 |
| 2.5 | Different mechanisms of anticancer action by naturally derived alkaloids | 21 |
| 2.6 | Overview of the cell cycle | 32 |
| 2.7 | Mechanism of action for well-known natural and semi-synthetic anticancer medicines regarding to their antioxidant abilities | 39 |
| 4.1 | Selected species of Melastomataceae used in this study | 55 |
| 4.2 | Yield (%, w/w) of crude hexane (CHE), ethyl acetate (CEE), and methanol (CME) and extracts prepared from selected species from the family Melastomataceae | 60 |
| 4.3 | Qualitative analysis of the phytochemical properties of crude methanol extracts (CME) of selected species from the family Melastomataceae | 61 |
| 4.4 | $IC_{50}$ values of crude extracts tested against human cancer cell lines | 63 |
| 4.5 | Selectivity of the cytotoxically active crude extracts of *M. caeruleum* and *P. rotundifolia* in comparison with MRC-5 cells | 65 |
| 4.6 | Identification of compounds in CEE of *M. caeruleum* and *P. rotundifolia* by using LC-MS/MS data | 67 |
| 4.7 | Free radical scavenging activity of crude extracts | 70 |
| 4.8 | Reducing potential of crude at different concentrations | 71 |

| | | |
|---|---|---|
| 4.9 | Total phenolic content of crude extracts at different concentrations | 72 |
| 4.10 | Antioxidative properties of crude extracts at 1.0 mg/ml | 74 |
| 4.11 | Pearson’s correlation coefficients (r) between total phenolic content and antioxidant capacity | 75 |

# LIST OF SYMBOLS AND ABBREVIATIONS

| | |
|---|---|
| -ve | Negative |
| +ve | Positive |
| °C | degree celcius |
| % | Percentage |
| µl | Microliter |
| µM | Micromole |
| µg/ml | microgram per millilitre |
| AIDS | Acquired Immunodeficiency Syndrome |
| ATCC | American Culture Collection |
| B.C | Before Century |
| c. | About |
| cell/ml | cell per millilitre |
| CEE | crude ethyl acetate extract |
| CHE | crude hexane extract |
| CME | crude methanol extract |
| $cm^3$ | cubic centimetre |
| $CO_2$ | carbon dioxide |
| DMEM | Dulbecco's Modified Eagle Medium |
| DMSO | Dimethylsulfoxide |
| DNA | deoxyribonucleic acid |
| DPPH | 2,2-diphenyl-1-picrylhydrazyl |
| EDTA | ethylene diamine tetra acetic acid |
| EtOAc | ethyl acetate |
| FBS | feotal bovine serum |
| F-C | Follin Ciocalteou reagent |
| $FeSO_4$ | ferrous sulphate |
| FRAP | ferric reducing antioxidant power |
| FTC | ferric thiocyanate |
| GACP | Good Agricultural and Collection Practices |
| GAE | Gallic Acid Equivalent |
| HEPES | N-2-Hydroxylethyl-Piperazine-N-2-Ethane-Sulfonoc |
| HIV | Human Immunodeficiency Virus |
| IC | inhibition concentration |
| LC-MS | liquid chromatography – mass spectrometry |
| LDL | low density lipoprotein |
| M | Meter |
| MEM | Minimum Essential Medium |
| MeOH | Methanol |
| Mg | milligram |
| mg/g | milligram per gram |
| Min | Minute |
| Mm | millimetre |
| Ml | millilitre |
| MS-MS | mass spectrometry |
| $Na_2CO_3$ | sodium carbonate |
| NADPH | nicotinamide adenine dinucleotide phosphate |
| Nm | nanometre |
| NR | neutral red |
| NRU | neutral red uptake assay |

| | |
|---|---|
| $O_2^{\cdot}$ | peroxide radical |
| OD | optical density |
| $^{\cdot}OH$ | hydroxyl radical |
| PBS | phosphate buffer saline |
| RNA | ribonucleic acid |
| ROS | reactive oxygen species |
| Rpm | rotation per minute |
| RPMI 1640 | Roswell Park Memorial Institute 1640 |
| S.D | standard deviation |
| SI | selectivity index |
| spp. | Species |
| TPC | total phenolic content |
| TPTZ | 2, 4, 6-tripyridyl-s-triazine |
| uHPLC | ultra high performance liquid chromatography |
| v/v | volume per volume |
| WHO | World Health Organization |
| w/v | weight per volume |

# LIST OF APPENDICES


| Appendix | | Page |
|---|---|---|
| 1 | Preparation of Solutions and Reagents | 112 |
| 2 | Cytotoxic Activity Raw Data | 121 |
| 3 | The Mass Spectrum of Detected Compounds in CEE of *M. caeruleum* and *P. rotundifolia* by LC-MS/MS | 124 |
| 4 | Antioxidant Potential Raw Data | 132 |