BIOASSAY-GUIDED ISOLATION AND IDENTIFICATION OF ANTIMICROBIAL AND CYTOTOXIC COMPOUNDS FROM THE LEAVES OF *Muntingia calabura*

ADILA SAHIDA BINTI SUFIAN

Thesis submitted in fulfilment of the requirements for the degree of

Master of Science

Faculty of Pharmacy

September 2013
I declare that the work in this thesis was carried out in accordance with the regulations of Universiti Teknologi MARA. It is original and is the result of my own work, unless otherwise indicated or acknowledged as reference work. This thesis has not been submitted to any other academic institution or non-academic institution for any degree or qualification.

I, hereby, acknowledge that I have been supplied with the Academic Rules and Regulations for Post Graduate, Universiti Teknologi MARA, regulating the conduct of my study and research.

Name of Student : Adila Sahida Binti Sufian

Student I.D. No : 2008262996

Programme : Master of Science

Faculty : Pharmacy

Thesis Title : Bioassay-guided Isolation and Identification of Antimicrobial and Cytotoxic Compounds from the Leaves of Muntingia calabura

Signature of Student : ............................................................

Date : September 2013
In the present work, isolation and identification of antimicrobial and cytotoxic compounds from the leaves of *Muntingia calabura* (Elaeocarpaceae) was carried out based on bioassay-guided approach. The methanol leaf extract of *M. calabura* (MCME) and its partitioned extracts [petroleum ether (MCPE), ethyl acetate (MCEA) and aqueous (MCAQ)] were subjected to antimicrobial and cytotoxic activities using micro-broth dilution and MTT assays, respectively. MCEA, which appeared to be the most active extract against MSSA (*S. aureus* 25923) (MIC = 125 µg/mL) and HL60 cell line (human acute promyelocytic leukemia) (IC$_{50}$ = 17.26 µg/mL), was further fractionated using vacuum liquid chromatography (VLC) to afford seven fractions (F1-F7). These fractions were again subjected to antimicrobial and cytotoxic activities. Purification of the most bioactive fraction, F5 with column chromatography (CC) and radial chromatography (RC) resulted in the isolation of four compounds (MC1-MC4). The structure of these compounds were elucidated by spectroscopic methods (1D-NMR, 2-D-NMR, UV, IR and MS) and compared with published data. MC1, MC2, MC3 and MC4 were identified as 5,7-dihydroxy-3,8-dimethoxyflavone, 2',4'-dihydroxychalcone, 5-hydroxy-3,7-dimethoxyflavone and 3,5,7-trihydroxy-8-methoxyflavone, respectively. Antimicrobial activity showed that MC2 exhibited the most significant activity against MSSA (MIC = 50 µg/mL) whereas, cytotoxic activity showed that MC2 and MC3 exhibited very strong activity against HL60 (IC$_{50}$ values of 3.43 and 3.34 µg/mL, respectively). The results clearly indicate that antimicrobial activity of *M. calabura* is ascribable to the presence of MC2. Meanwhile, cytotoxic activity of *M. calabura* is ascribable to the presence of MC2 and MC3. The leaves of *M. calabura* show promise as a source of antimicrobial and anticancer agents and warrant further exploration.
# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>AUTHOR'S DECLARATION</td>
<td>ii</td>
</tr>
<tr>
<td>ABSTRACT</td>
<td>iii</td>
</tr>
<tr>
<td>ACKNOWLEDGMENTS</td>
<td>iv</td>
</tr>
<tr>
<td>TABLE OF CONTENTS</td>
<td>v</td>
</tr>
<tr>
<td>LIST OF TABLES</td>
<td>viii</td>
</tr>
<tr>
<td>LIST OF FIGURES</td>
<td>ix</td>
</tr>
<tr>
<td>LIST OF SYMBOLS</td>
<td>xi</td>
</tr>
<tr>
<td>LIST OF ABBREVIATIONS</td>
<td>xii</td>
</tr>
</tbody>
</table>

**CHAPTER ONE: INTRODUCTION**

1.1 Background 1  
1.2 Problem Statement 2  
1.3 Significance of Study 2  
1.4 Scope of Research 3  
1.5 Objectives of Research 3

**CHAPTER TWO: LITERATURE REVIEW**

2.1 Infectious Disease 4  
2.2 Antibiotics 4  
2.2.1 Antibiotics Resistance 5  
2.3 Cancer 6  
2.4 Natural Products from Plants 6  
2.4.1 Plants as a Source of Antimicrobial Agents 7  
2.4.2 Plants as a Source of Anticancer Agents 7  
2.5 Flavonoids 8  
2.5.1 Antimicrobial Activity of Flavonoids 8  
2.5.2 Cytotoxic Activity of Flavonoids 9  
2.6 The Plant: *Muntingia calabura* 10  
2.6.1 Morphological and Botanical Description 10
CHAPTER THREE: RESEARCH METHODOLOGY

3.1 General Instrumentation 25
3.2 Chromatographic Techniques 25
3.3 Solvents and Reagents 25
3.4 Phytochemical Screening 26
   3.4.1 Test for Flavonoids 26
   3.4.2 Test for Alkaloids 26
   3.4.3 Test for Saponins 26
   3.4.4 Test for Tannins 27
3.5 Flavonoid Detection 27
3.6 Plant Materials 27
3.7 Extraction and Partition Procedures 27
3.8 Bioassay-guided Fractionation and Isolation of Bioactive Compound(s) 28
3.9 Bioassay Procedure: Antimicrobial Activity 31
   3.9.1 Microbial Strains 31
   3.9.2 Determination of MIC 31
   3.9.3 Determination of MBC and MFC 32
3.10 Bioassay Procedure: Cytotoxic Activity 33
   3.10.1 Cell Lines and Cell Conditions 33
   3.10.2 MTT Assay 33
   3.10.3 Statistical Analysis 36

CHAPTER FOUR: RESULTS AND DISCUSSION

4.1 Preliminary Screening 37
   4.1.1 Phytochemical Screening 37
   4.1.2 Antimicrobial Activity of M. calabura extracts 37
   4.1.3 Cytotoxic Activity of M. calabura extracts 39
4.2 Bioassay-guided Fractionation of Ethyl Acetate Leaf Extract of M. calabura (MCEA) 41