ANTIOXIDANT ACTIVITIES AND INHIBITORY EFFECTS OF Mikania micrantha Kunth (SELAPUT TUNGGUL) AGAINST KEY ENZYMES INVOLVED IN HYPERLIPIDEMIA AND HYPERTENSION IN VITRO

AMIRAH HAZIYAH BINTI ISHAK

FPSK(M) 2018 38
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By

AMIRAH HAZIYAH BINTI ISHAK

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

April 2018
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ANTIOXIDANT ACTIVITIES AND INHIBITORY EFFECTS OF Mikania micrantha Kunth (SELAPUT TUNGGUL) AGAINST KEY ENZYMES INVOLVED IN HYPERLIPIDEMIA AND HYPERTENSION IN VITRO

By

AMIRAH HAZIYAH BINTI ISHAK

April 2018

Chairman : Nurul Husna Shafie, PhD
Faculty : Medicine and Health Sciences

Mikania micrantha Kunth, locally known as ‘Selaput tunggul’ in Malaysia, is a plant that traditionally used to reduce the risk of diabetes, hypercholesterolemia, and hypertension. This study was aimed to investigate the antioxidant capacities and inhibitory activities of various extracts of the leaves and stems of M. micrantha on key enzymes related to hyperlipidemia and hypertension in vitro.

Total phenolic content (TPC) and total flavonoid content (TFC) were determined using the Folin-Ciocalteu and aluminium chloride colorimetric assays, respectively. The antioxidant capacities were determined using 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging, 2,2’-azinobis-3-ethylbenzothiazoline-6-sulphonic acid (ABTS), ferric reducing antioxidant power (FRAP), phosphomolybdenum antioxidative power (PAP), and β-carotene bleaching (BCB) assays. The inhibitory activities of M. micrantha on pancreatic lipase (PL), lipoprotein lipase (LPL), HMG-CoA reductase (HMGR), and angiotensin-converting enzyme (ACE) were evaluated in vitro using the spectrophotometric method. In addition, the chemical profiling of the selected extracts was determined using gas chromatography-mass spectrometry (GC-MS).

The results demonstrated the ethyl acetate stems (EAS) and leaves (EAL) extracts of M. micrantha had significantly (p < 0.05) greatest TPC (141±0.51 mg gallic acid equivalent/g) and TFC (70.1±0.92 catechin equivalent/g), respectively, compared to samples extracted by other solvents. The EAS extract had also significantly greatest antioxidant capacities using DPPH (EC\textsubscript{50}=324±61.4 μg/mL), ABTS (0.53±0.01 mmol trolox equivalent/g), FRAP
(1.28±0.05 mmol Fe²⁺/g), PAP (219±7.03 mg ascorbic acid equivalent/g), and BCB (108±2.23%) assays.

The ethanol stems (ETS) extract exhibited the highest PL inhibitory activity (IC₅₀=4.49±2.50 μg/mL) followed by hot water leaves (HWL; IC₅₀=4.56±0.07 μg/mL) and ethanol leaves (ETL; IC₅₀=8.02±1.56 μg/mL). These extracts also showed no significant (p > 0.05) difference between each other and orlistat (IC₅₀=0.31±0.01 μg/mL). The ethanol leaves (ETL) extract showed the highest LPL inhibitory activity (IC₅₀=1.42±0.48 μg/mL), however, the difference was found not significant (p > 0.05) between all extracts and orlistat (IC₅₀=1.98±1.22 μg/mL). ETL also showed the highest inhibitory activity against HMG-CoA reductase (50.12±3.44% inhibition), but not significant (p > 0.05) when compared to other extracts except hot water stems (HWS) extract. HWS extract showed the least inhibitory activity against PL, LPL, and HMG-CoA reductase. However, HWS extract showed the greatest ACE inhibition (97.47±1.19%), but not significantly (p > 0.05) different when compared to other extracts and captopril (98.42±0.93%). Overall, all extracts exhibited remarkable inhibitory activity against PL, LPL, HMG, and ACE.

GC-MS analysis of EAL and EAS extracts showed the presence of sesquiterpenes (18.74% and 30.46%, respectively), phenol (14.74% and 16.38%, respectively), and alkane hydrocarbons (26.7% and 10.45%, respectively) which might contribute to its antioxidant and enzyme inhibitory activities. In conclusion, this study indicates the potential of ethyl acetate stems, ethanol leaves, and ethanol stems extracts as antioxidant, anti-hyperlipidemic and anti-hypertensive agents. Results from this study provide baseline knowledge and evidence of the traditional uses of *M. micrantha* which could be the guidance for future development of nutraceuticals from *M. micrantha* for hyperlipidemia and hypertension.
Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains

AKTIVITI ANTIOKSIDAN DAN KESAN PPERENCATAN Mikania micrantha Kunth (SELAPUT TUNGGUL) TERHADAP ENZIM UTAMA YANG TERLIBAT DALAM HIPERLIPIDEMIA DAN HIPERTENSI IN VITRO

Oleh

AMIRAH HAZIYAH BINTI ISHAK

April 2018

Pengerusi : Nurul Husna Shafie, PhD
Fakulti : Perubatan dan Sains Kesihatan

Mikania micrantha Kunth, yang dikenali sebagai 'Selaput tunggul' di Malaysia, merupakan sejenis tumbuhan yang digunakan secara tradisional untuk mengurangkan risiko diabetes, hiperkolesterolemia, dan hipertensi. Kajian ini bertujuan untuk mengkaji keupayaan antioksidan dan aktiviti perencatan pelbagai ekstrak daripada bagian daun dan batang pokok Mikania micrantha terhadap enzim utama yang terlibat dalam hiperlipidemia dan hipertensi secara in vitro.

Jumlah kandungan fenolik (TPC) dan jumlah kandungan flavonoid (TFC) masing-masing ditentukan menggunakan kaedah Folin-Ciocalteu dan aluminium klorida kolorimetri. Kapasiti antioksidan telah ditentukan menggunakan kaedah perencatan radikal 2,2-difenil-1-pikrilhidrazil (DPPH), asid 2,2'-azinobis-3-etylbenzothiazoline-6-sulfonat (ABTS), kuasa antioksidan penurunan ferik (FRAP), kuasa antioksidatif phosphomolybdenum (PAP) dan pelunturan β-karotena (BCB). Aktiviti perencatan Mikania micrantha pada lipase pankreas (PL), lipase lipoprotein (LPL), HMG-CoA reductase (HMG), dan enzim penukar angiotensin (ACE) telah dinilai secara in vitro menggunakan kaedah spektrofotometri. Sebagai tambahan, profil kimia dari ekstrak yang dipilih ditentukan menggunakan gas kromatografi-spektrometri jisim (GC-MS).

Hasil kajian menunjukkan ekstrak etil asetat daripada batang (EAS) dan daun (EAL) Mikania micrantha mempunyai kandungan TPC (141±0.51 mg setara asid galik/g) dan TFC (70.1±0.92 mg setara catechin/g) yang ketara (p < 0.05) tinggi berbanding dengan sampel yang diekstrak menggunakan pelarut lain. Ekstrak EAS juga mempunyai kapasiti antioksidan tertinggi apabila diukur menggunakan kaedah DPPH (EC50=324±61.4 μg/mL), ABTS (0.53±0.01 mmol
setara trolok/g), FRAP (1.28±0.05 mmol Fe²⁺/g), PAP (219±7.03 mg setara asid askorbik/g), dan BCB (108 ± 2.23%).

Ekstrak etanol daripada batang (ETS) menunjukkan aktiviti perencatan PL tertinggi (IC₅₀=4.49±2.50 μg/mL) diikuti oleh ekstrak air panas daripada daun (HWL; IC₅₀=4.56±0.07 μg/mL) dan etanol daun (ETL; IC₅₀=8.02±1.56 μg/mL). Ekstrak ini juga menunjukkan tiada perbezaan ketara (p > 0.05) di antara satu sama lain dan juga orlistat (IC₅₀=0.31 ± 0.01 μg/mL). Ekstrak etanol daripada daun (ETL) menunjukkan aktiviti perencatan LPL tertinggi (IC₅₀=1.42±0.48 μg/mL), bagaimanapun, perbezaan di antara semua ekstrak dan orlistat (IC₅₀=1.98±1.22 μg/mL) adalah tidak ketara. ETL juga menunjukkan aktiviti perencatan tertinggi terhadap HMG-CoA reduktase (50.12±3.44%), tetapi tiada perbezaan ketara berbanding ekstrak yang lain kecuali ekstrak air panas daripada batang (HWS). Ekstrak HWS menunjukkan aktiviti perencatan yang terendah terhadap PL, LPL, dan HMG-CoA reduktase. Namun begitu, ekstrak HWS menunjukkan perencatan ACE yang tertinggi, walaupun tiada perbezaan ketara berbanding ekstrak lain dan juga captopril (98.42±0.93%). Keseluruhannya, semua ekstrak menunjukkan aktiviti perencatan menentang PL, LPL, HMGR, dan ACE.

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I certify that a Thesis Examination Committee has met on 19 April 2018 to conduct the final examination of Amirah Haziyah binti Ishak on her thesis entitled “Antioxidant Activities and Inhibitory Effects of Mikania micrantha Kunth (Selaput Tunggul) Against Key Enzymes Involved in Hyperlipidemia and Hypertension In Vitro.” in accordance with the Universities and University Colleges Act 1971 and the Consultation 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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Date: 28 June 2018
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<th>Signature</th>
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</tr>
</thead>
<tbody>
<tr>
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</table>

<table>
<thead>
<tr>
<th>Signature</th>
<th>Name of Member of Supervisory Committee</th>
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<tbody>
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<td>Assoc. Prof. Dr. Norhaizan Mohd Esa</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Signature</th>
<th>Name of Member of Supervisory Committee</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Dr. Hasnah Bahari</td>
</tr>
</tbody>
</table>
# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>ABSTRACT</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>ABSTRAK</td>
<td>iii</td>
</tr>
<tr>
<td>ACKNOWLEDGEMENT</td>
<td>v</td>
</tr>
<tr>
<td>APPROVAL</td>
<td>vi</td>
</tr>
<tr>
<td>DECLARATION</td>
<td>viii</td>
</tr>
<tr>
<td>LIST OF TABLES</td>
<td>xiii</td>
</tr>
<tr>
<td>LIST OF FIGURES</td>
<td>xiv</td>
</tr>
<tr>
<td>LIST OF ABBREVIATIONS</td>
<td>xv</td>
</tr>
</tbody>
</table>

## CHAPTER

1 **INTRODUCTION**
1.1 Research background 1
1.2 Problem statement 3
1.3 Significance of study 4
1.4 Research objectives 4
1.5 Null hypothesis 5

2 **LITERATURE REVIEW**
2.1 Cardiovascular diseases (CVD)
  2.1.1 Atherosclerosis 6
  2.1.2 Endothelial dysfunction 6
2.2 Oxidative stress and antioxidants 7
2.3 Cholesterol and lipoproteins 10
2.4 Cholesterol biosynthesis 10
2.5 Hyperlipidemia
  2.5.1 Prevalence of hyperlipidemia 12
  2.5.2 Enzymes related to hyperlipidemia 13
  2.5.3 Current treatment for hyperlipidemia 14
2.6 Hypertension
  2.6.1 Prevalence of hypertension 16
  2.6.2 Enzymes related to hypertension 18
  2.6.3 Current treatment for hypertension 19
2.7 Antioxidant, anti-hyperlipidemic and antihypertensive agents from other natural sources
  2.7.1 Effects of different types of solvents on antioxidant properties 21
2.8 *Mikania micrantha* Kunth
  2.8.1 Traditional medicinal uses of *M. micrantha* 24
  2.8.2 Chemical constituents of *M. micrantha* 25
  2.8.3 Pharmacological properties of *M. micrantha* 26
3 MATERIALS AND METHODS

3.1 Chemical and reagents 29
3.2 Sample collection and preparation 29
3.3 Extraction of M. micrantha 29
3.4 Determination of antioxidant contents 30
  3.4.1 Total phenolic contents (TPC) 30
  3.4.2 Total flavonoid contents (TFC) 31
3.5 Determination of antioxidant capacities 31
  3.5.1 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay 31
  3.5.2 2,2’-azinobis-3-ethylbenzothiazoline-6-sulphonic acid (ABTS) assay 31
  3.5.3 Ferric reducing antioxidant power (FRAP) assay 32
  3.5.4 Phosphomolybdenum antioxidative power (PAP) assay 32
  3.5.5 β-carotene bleaching (BCB) assay 33
3.6 Gas chromatography-mass spectrometry (GC-MS) analysis 33
3.7 Enzyme assays 34
  3.7.1 Pancreatic lipase (PL) inhibition assay 34
  3.7.2 Lipoprotein lipase (LPL) inhibition assay 34
  3.7.3 HMG-CoA reductase (HMGR) inhibition assay 35
  3.7.4 Angiotensin-I converting enzyme (ACE) inhibition assay 35
3.8 Statistical analysis 36

4 RESULTS AND DISCUSSION

4.1 Extraction yield of M. micrantha extracts 37
4.2 Antioxidant contents 38
  4.2.1 Total phenolic contents (TPC) 39
  4.2.2 Total flavonoid contents (TFC) 40
4.3 Antioxidant capacities 42
  4.3.1 DPPH radical scavenging capacity 42
  4.3.2 ABTS radical scavenging capacity 45
  4.3.3 Ferric reducing antioxidant power (FRAP) assay 47
  4.3.4 Phosphomolybdenum antioxidative power (PAP) assay 48
  4.3.5 β-carotene bleaching (BCB) assay 50
4.4 Correlation between antioxidant contents and antioxidant capacities 52
4.5 GC-MS analysis 54
4.6 Enzyme assays 58
  4.6.1 Pancreatic lipase (PL) inhibition assay 59
  4.6.2 Lipoprotein lipase (LPL) inhibition assay 62
  4.6.3 HMG-CoA reductase (HMGR) inhibition assay 64
  4.6.4 Angiotensin-I converting enzyme (ACE) inhibition assay 67
4.7 Correlation between TPC, TFC, and enzyme inhibition 69
# LIST OF TABLES

<table>
<thead>
<tr>
<th>Table</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.1</td>
<td>Current therapeutic agents for treatment of hyperlipidemia</td>
</tr>
<tr>
<td>2.2</td>
<td>Current therapeutic agents for treatment of hypertension</td>
</tr>
<tr>
<td>4.1</td>
<td>DPPH radical scavenging capacity of <em>M. micrantha</em> extracts</td>
</tr>
<tr>
<td>4.2</td>
<td>Pearson correlation analysis of the antioxidant contents and the antioxidant capacities of <em>M. micrantha</em> extracts</td>
</tr>
<tr>
<td>4.3</td>
<td>Phytochemicals identified in the leaves and stems of <em>M. micrantha</em> extracted using ethyl acetate</td>
</tr>
<tr>
<td>4.4</td>
<td>IC\textsubscript{50} values for pancreatic lipase inhibitory activity of <em>M. micrantha</em> extracts</td>
</tr>
<tr>
<td>4.5</td>
<td>IC\textsubscript{50} values for lipoprotein lipase inhibitory activity of <em>M. micrantha</em> extracts</td>
</tr>
<tr>
<td>4.6</td>
<td>Pearson correlation analysis of the antioxidant contents and enzymes inhibitory activity of <em>M. micrantha</em></td>
</tr>
</tbody>
</table>
## LIST OF FIGURES

<table>
<thead>
<tr>
<th>Figure</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.1</td>
<td>Classification of antioxidants</td>
</tr>
<tr>
<td>2.2</td>
<td>Simplified cholesterol biosynthesis pathway</td>
</tr>
<tr>
<td>2.3</td>
<td>Simplified diagram for the renin-angiotensin system (RAS)</td>
</tr>
<tr>
<td>2.4</td>
<td>Herbarium sheet of whole plant parts of <em>M. micrantha</em> (a), the inflorescences (b), and stems (c) of <em>M. micrantha</em></td>
</tr>
<tr>
<td>4.1</td>
<td>Extraction yield of the leaves and stems of <em>M. micrantha</em> using different solvents</td>
</tr>
<tr>
<td>4.2</td>
<td>Total phenolic content (TPC) of various <em>M. micrantha</em> extracts</td>
</tr>
<tr>
<td>4.3</td>
<td>Total flavonoid content (TFC) of various <em>M. micrantha</em> extracts</td>
</tr>
<tr>
<td>4.4</td>
<td>Percentage of DPPH radical scavenging activities of (a) <em>M. micrantha</em> leaves extracts and (b) <em>M. micrantha</em> stems extracts in comparison with the standard (BHT)</td>
</tr>
<tr>
<td>4.5</td>
<td>ABTS radical scavenging capacity of various <em>M. micrantha</em> extracts</td>
</tr>
<tr>
<td>4.6</td>
<td>FRAP value of various <em>M. micrantha</em> extracts</td>
</tr>
<tr>
<td>4.7</td>
<td>PAP value of various <em>M. micrantha</em> extracts</td>
</tr>
<tr>
<td>4.8</td>
<td>BCB value of various <em>M. micrantha</em> extracts</td>
</tr>
<tr>
<td>4.9</td>
<td>GC-MS chromatogram for (a) EAL and (b) EAS extracts of <em>M. micrantha</em></td>
</tr>
<tr>
<td>4.10</td>
<td>Dose-response curve of pancreatic lipase inhibitory activity for the (a) leaves and (b) stems of <em>M. micrantha</em>.</td>
</tr>
<tr>
<td>4.11</td>
<td>Dose-response curve of lipoprotein lipase inhibitory activity for the (a) leaves and (b) stems of <em>M. micrantha</em>.</td>
</tr>
<tr>
<td>4.12</td>
<td>HMGR inhibitory activity of <em>M. micrantha</em> extracts</td>
</tr>
<tr>
<td>4.13</td>
<td>ACE inhibitory activity of <em>M. micrantha</em> extracts</td>
</tr>
<tr>
<td>4.14</td>
<td>Schematic mechanism of action of <em>M. micrantha</em> as antioxidant, anti-hyperlipidemic and anti-hypertensive agents</td>
</tr>
</tbody>
</table>
### LIST OF ABBREVIATIONS

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Full Form</th>
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</tr>
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<td>Ascorbic acid equivalent</td>
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<td></td>
</tr>
<tr>
<td>EAL</td>
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<td></td>
</tr>
<tr>
<td>EAS</td>
<td>Ethyl acetate stems</td>
<td></td>
</tr>
<tr>
<td>ETL</td>
<td>Ethanol leaves</td>
<td></td>
</tr>
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<td>Ethanol stems</td>
<td></td>
</tr>
<tr>
<td>FRAP</td>
<td>Ferric reducing antioxidant power</td>
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</tr>
<tr>
<td>GAE</td>
<td>Gallic acid equivalent</td>
<td></td>
</tr>
<tr>
<td>GC-MS</td>
<td>Gas chromatography-mass spectrometry</td>
<td></td>
</tr>
<tr>
<td>GSH</td>
<td>Glutathione</td>
<td></td>
</tr>
<tr>
<td>GSHPx</td>
<td>Glutathione peroxidase</td>
<td></td>
</tr>
<tr>
<td>HA</td>
<td>Hippuric acid</td>
<td></td>
</tr>
<tr>
<td>HAT</td>
<td>Hydrogen atom transfer</td>
<td></td>
</tr>
<tr>
<td>HDL</td>
<td>High-density lipoprotein</td>
<td></td>
</tr>
<tr>
<td>HDL-C</td>
<td>High-density lipoprotein cholesterol</td>
<td></td>
</tr>
<tr>
<td>HHL</td>
<td>Hippuryl-histidyl-leucine</td>
<td></td>
</tr>
<tr>
<td>HMG-CoA</td>
<td>3-hydroxy-3-methylglutaryl coenzyme A</td>
<td></td>
</tr>
<tr>
<td>HMGR</td>
<td>HMG-CoA reductase</td>
<td></td>
</tr>
<tr>
<td>HPLC</td>
<td>High-performance liquid chromatography</td>
<td></td>
</tr>
<tr>
<td>HWL</td>
<td>Hot water leaves</td>
<td></td>
</tr>
<tr>
<td>HWS</td>
<td>Hot water stems</td>
<td></td>
</tr>
<tr>
<td>HXL</td>
<td>Hexane leaves</td>
<td></td>
</tr>
<tr>
<td>HXS</td>
<td>Hexane stems</td>
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</tr>
<tr>
<td>IDL</td>
<td>Intermediate density lipoprotein</td>
<td></td>
</tr>
<tr>
<td>LDL-C</td>
<td>Low-density lipoprotein cholesterol</td>
<td></td>
</tr>
<tr>
<td>LPL</td>
<td>Lipoprotein lipase</td>
<td></td>
</tr>
<tr>
<td>M. micrantha</td>
<td>Mikania micrantha</td>
<td></td>
</tr>
<tr>
<td>Acronym</td>
<td>Description</td>
<td></td>
</tr>
<tr>
<td>---------</td>
<td>-------------</td>
<td></td>
</tr>
<tr>
<td>MW</td>
<td>Molecular weight</td>
<td></td>
</tr>
<tr>
<td>NCD</td>
<td>Non-communicable diseases</td>
<td></td>
</tr>
<tr>
<td>NHMS</td>
<td>National Health Morbidity Survey</td>
<td></td>
</tr>
<tr>
<td>NO</td>
<td>Nitric oxide</td>
<td></td>
</tr>
<tr>
<td>OGTT</td>
<td>Oral glucose tolerance test</td>
<td></td>
</tr>
<tr>
<td>ox-LDL</td>
<td>Oxidized low-density lipoprotein</td>
<td></td>
</tr>
<tr>
<td>PAP</td>
<td>Phosphomolybdenum antioxidative power</td>
<td></td>
</tr>
<tr>
<td>PL</td>
<td>Pancreatic lipase</td>
<td></td>
</tr>
<tr>
<td>ρ-NPB</td>
<td>p-nitrophenylbutyrate</td>
<td></td>
</tr>
<tr>
<td>PPL</td>
<td>Porcine pancreatic lipase</td>
<td></td>
</tr>
<tr>
<td>ppm</td>
<td>Part per million</td>
<td></td>
</tr>
<tr>
<td>RAAS</td>
<td>Renin-angiotensin-aldosterone system</td>
<td></td>
</tr>
<tr>
<td>RAS</td>
<td>Renin-angiotensin system</td>
<td></td>
</tr>
<tr>
<td>ROS</td>
<td>Reactive oxygen species</td>
<td></td>
</tr>
<tr>
<td>RT</td>
<td>Retention time</td>
<td></td>
</tr>
<tr>
<td>SEM</td>
<td>Standard error of mean</td>
<td></td>
</tr>
<tr>
<td>SMC</td>
<td>Smooth muscle cell</td>
<td></td>
</tr>
<tr>
<td>SOD</td>
<td>Superoxide dismutase</td>
<td></td>
</tr>
<tr>
<td>TE</td>
<td>Trolox equivalent</td>
<td></td>
</tr>
<tr>
<td>TEAC</td>
<td>Trolox equivalent antioxidant capacity</td>
<td></td>
</tr>
<tr>
<td>TFC</td>
<td>Total flavonoid contents</td>
<td></td>
</tr>
<tr>
<td>TG</td>
<td>Triglycerides</td>
<td></td>
</tr>
<tr>
<td>TPC</td>
<td>Total phenolic contents</td>
<td></td>
</tr>
<tr>
<td>VLDL</td>
<td>Very low-density lipoprotein</td>
<td></td>
</tr>
<tr>
<td>VLDL-C</td>
<td>Very low-density lipoprotein cholesterol</td>
<td></td>
</tr>
<tr>
<td>WHO</td>
<td>World Health Organization</td>
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</table>
CHAPTER 1

INTRODUCTION

1.1 Research background

The importance of plants to human life can be seen in its diverse utilization in medicine and food as nutraceuticals. Plants are a large source of new bioactive molecules with therapeutic potentials and they have increasingly become attractive alternatives to prevent or reduce risk factors of cardiovascular diseases (CVD) such as hyperlipidemia and hypertension (Nasri et al., 2014). Phytomedicines may benefit the human healthcare systems since they contain many free radical scavenging molecules such as phenolic compounds, nitrogen compounds, vitamins, terpenoids and other compounds that exhibit antioxidant properties (Chetan et al., 2012). The bioactive compounds of the plants will contribute to their medicinal value which produces definite physiological actions on the human body (Hasmida et al., 2014). Moreover, there is evidence suggesting that natural antioxidants from plant extracts are safe and they may reduce the risk of non-communicable diseases (NCD) without any side effects (Chetan et al., 2012).

Dyslipidemia refers to an abnormal amount of cholesterol and triglycerides level in the blood. Hyperlipidemia, hypertriglyceridemia, and hypercholesterolemia are the types of dyslipidemia resulting from the elevation of both cholesterol and triglycerides (Moor et al., 2017). Hypercholesterolemia and hypertension are the important components of metabolic syndromes and risk factors for CVD. The presence of these two risk factors could lead to the development of atherosclerosis and consequently to CVD (Dalal et al., 2012). Hypercholesterolemia is a disease condition that is characterized by an abnormally increased level of plasma lipoproteins; particularly the low-density lipoprotein cholesterol (LDL-C). The deposition of LDL-C in the lining of vascular wall will develop atherosclerotic plaque thus narrowing the diameter of the blood vessel (Alinde et al., 2012). Moreover, oxidized LDL inhibits endothelial nitric oxide (NO) formation, which contributes to artery stiffening, thereby causing hypertension (Sander & Giles, 2002). Hypertension is defined as persistent elevation of systolic and diastolic blood pressure (BP) that exceeds 140 and 90 mmHg, respectively. Angiotensin II, a hormone which is strongly related to hypertension, triggers endothelial dysfunction in hypertensive patients (John & Schmieder, 2003).

A combination of different strategies is used to treat and manage hyperlipidemia and hypertension. Common therapeutic strategies are generally directed at lowering the serum LDL-C levels and prevention of angiotensin II formation through the inhibition of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase and angiotensin-I converting enzyme (ACE) which are
the key enzymes of hypercholesterolemia and hypertension, respectively (Ademosun et al., 2015; Saravanan & Ignacimuthu, 2015; Martinello et al., 2006). Other strategies include the inhibition of pancreatic lipase (PL) and lipoprotein lipase (LPL) which are the key enzymes in lipid metabolism responsible for hydrolysis of dietary fats in the intestine and lipolysis of triglycerides in lipoprotein, respectively (Li et al., 2014). Inhibition of both PL and LPL can help to reduce dietary triglycerides absorption, hence, decrease the absorption of dietary cholesterol.

There are reports of established lipases, HMG-CoA reductase and ACE inhibitors such as orlistat (Alqahtani et al., 2015), statins (Chogtu et al., 2015), and captopril (Weber et al., 2014), respectively. Lipase inhibitor drug such as orlistat decreases the absorption of dietary cholesterol by inhibition of intestinal lipases (Alqahtani et al., 2015). However, orlistat gives side effects such as steatorrhoea, bloating, oily spotting, faecal urgency, faecal incontinence and even caused subacute liver failure (Drew et al., 2007; Thurairajah et al., 2005). Modern drugs such as statins, fibrates, nicotinic acid, and resins have been used to lower blood cholesterol level by inhibiting the endogenous synthesis or by lowering the cholesterol absorption from the intestine (Saravanan & Ignacimuthu, 2015). However, the high cost for synthetic drugs and the remaining side effects such as distal muscle weakness, headache, acute renal failure, polyneuropathy, memory loss, sleep disturbances, impotence, and pancreatitis lead to the findings for low cost remedies from natural resources (Lin et al., 2015; Djerrou, 2014). Statins have been widely used for the treatment of hypercholesterolemia but it was found that they could also slightly increase the potential risk of type 2 diabetes (Sattar et al., 2010), necessitating the search for more options of no or less adverse effects of hypercholesterolemia treatments. Meanwhile, ACE inhibitors such as captopril give side effects such as proteinuria, skin rashes, altered taste (Atlas, 2007) and also cough (Sweitzer, 2003).

Nowadays, there are increasing interests for substitution of modern medicine with traditional plants and some natural component in the plants for treatment of hyperlipidemia and hypertension. According to Cheurfa and Allem (2015), plant-derived products are commonly considered to be less toxic, with few or no side effect than their synthetic equivalents. Mikania micrantha Kunth (Asteraceae or Compositae) is a perennial creeping vine and widely distributed in South and North America and can also be found in Africa, Pacific Islands and Southeast Asia, including Southern China and Malaysia (Day et al., 2016; Tripathi et al., 2012). This plant is known as American rope, Chinese creeper, mile-a-minute, ‘Chhagalbati’ or ‘Japanilata’ (West Bengal), ‘Selaput tunggul’ (Malaysia) and ‘Sembung rambat’ (Indonesia) (Saha et al., 2015; Haisya et al., 2013; Nornasuha & Ismail, 2013). In agriculture, M. micrantha is known as a weed plant that can reduce the growth and productivity of several crops such as rubber, oil palm, and cocoa plantation in Malaysia which cost 8-10 million dollars per annum to control its growth (Sankaran, 2008). This is due to its fast-growing habit and production of allelopathic substances (Day et al., 2016; Nornasuha & Ismail, 2013; Sankaran, 2008). However, this plant is used
traditionally to treat insect bites and stop minor external bleeding (Facey et al., 2010) or consumed as a juice as an alternative to reduce glucose, cholesterol, and high blood pressure.

*M. micrantha* has demonstrated many health benefits, such as antimicrobial (Chetia et al., 2014; Chetan et al., 2012; Facey et al., 2010), anti-diabetic (Wan Nurhayati et al., 2013), anti-dermatophytic (Jyothilakshmi et al., 2015), anti-stress (Sibi & Sajid, 2014), anti-inflammatory (Pérez-Amador et al., 2010), anti-proliferative (Ríos et al., 2014), and anti-cancer (Matawali et al., 2016; Dou et al., 2014) activities. In fact, *M. micrantha* is rich in phytochemicals such as terpenoids (sesquiterpene lactones), alkaloids, flavonoids, steroids, reducing sugars, saponins, phenolics, and tannins which have shown significant bioactivities (Dong et al., 2017; Dev et al., 2015).

In spite of its medicinal benefits as aforementioned, the information of the potential antioxidant agent in this plant is still scarce especially the comparison between different parts and the effect of different solvents which can affect the antioxidant capacities and bioactive compounds in *M. micrantha*. Moreover, the potential of *M. micrantha* as pancreatic lipase, lipoprotein lipase, HMG-CoA reductase and ACE inhibitors which are the key enzymes for hyperlipidemia and hypertension has not yet been investigated. Antioxidant agents are important to prevent oxidative damage-related diseases *i.e.*, cancer, cardiovascular diseases, diabetes, etc., (Sharma et al., 2014) thus potential health benefits of this plant should be further explored.

### 1.2 Problem statement

Non-communicable diseases (NCD) status in Malaysia such as CVD is in an alarming condition. This problem has significant implications on healthcare costs and drives increasing demand for relatively expensive treatment and long-term rehabilitative care (Mustapha et al., 2014). Hyperlipidemia and hypertension are frequently associated, and they might contribute to the development of atherosclerosis which consequently caused CVD (Dalal et al., 2012). The treatment and management of hyperlipidemia and hypertension include dietary changes and also the use of commercial drugs. However, these oral medications have certain limitations and side effects (Alqahtani et al., 2015; Chogtu et al., 2015; Weber et al., 2014).

Due to the side effects of commercially available drugs, the research on alternative treatment such as the use of medicinal plants needs to be studied. Medicinal plants have been used in traditional healthcare since decades and received much attention in recent years in the search of new therapeutic agents due to the low cost, easy availability, and less or no side effects (Sofowora et al., 2013). Based on previous studies, *M. micrantha* has demonstrated the potential therapeutic effects of several diseases including...
diabetes, infections, and cancers (Dou et al., 2014; Wan Nurhayati et al., 2013). Many phytochemicals have been detected from different parts of *M. micrantha* which contribute to the medicinal properties of this plant, especially terpenoids, the major compounds isolated (Nicollier & Thompson, 1981). However, further researches are needed to support the traditional claims and the limited scientific evidence on the health benefit of *M. micrantha*.

### 1.3 Significance of study

To date, there is limited information about the antioxidant capacities, anti-hyperlipidemic, and anti-hypertensive effects of various extracts of the leaves and stems of *M. micrantha*. Hence, results from this study could provide added values such as information on the best extraction solvent, parts of the plants and the phytochemicals present in *M. micrantha* and scientific evidence to prove the traditional medical uses of *M. micrantha*. In future, findings from the antioxidant and enzymatic inhibitory activities of *M. micrantha* extracts *in vitro* could serve as a basis for future *in vivo* research. *M. micrantha* may be a promising source in the search of new anti-hyperlipidemic and anti-hypertensive agents due to its efficacy and broad phytochemical range.

### 1.4 Research objectives

The general objective of this study is to determine the antioxidant capacities and potential inhibitory activities of various solvent extracts (hot water, cold water, 70% ethanol, and ethyl acetate) of the leaves and stems of *M. micrantha* on key enzymes related to hyperlipidemia and hypertension *in vitro*.

The specific objectives of the study are:

i. To determine and compare the total phenolic content (TPC), total flavonoid content (TFC), and antioxidant capacities of various solvent extracts of the leaves and stems of *M. micrantha* by 2,2’-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging, 2,2’-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid (ABTS) radical scavenging, ferric reducing antioxidant power (FRAP), phosphomolybdenum antioxidative power (PAP), and β-carotene bleaching (BCB) assays.

ii. To determine the phytochemicals of the leaves and stems of *M. micrantha* using gas chromatography-mass spectrometry (GC-MS) analysis.

iii. To determine the inhibitory activities of various solvent extracts of the leaves and stems of *M. micrantha* against lipases, *i.e.*, pancreatic lipase (PL) and lipoprotein lipase (LPL), HMG-CoA reductase (HMGCR) and angiotensin-I converting enzyme (ACE).
iv. To evaluate the correlation between antioxidant contents (TPC and TFC) with antioxidant capacities (DPPH, ABTS, FRAP, PAP, and BCB) and enzymes inhibitory activities (PL, LPL, HMGR, ACE) of *M. micrantha* extracts.

1.4 Null hypotheses

H₀ 1: There is no significant difference between the TPC, TFC, and antioxidant capacities of various solvent extracts of the leaves and stems of *M. micrantha*.

H₀ 2: There is no significant difference between the phytochemicals present in the leaves and stems of *M. micrantha*.

H₀ 3: There is no significant difference between the inhibitory activities of various solvent extracts of the leaves and stems of *M. micrantha* against PL, LPL, HMG-CoA reductase and ACE.

H₀ 4: There is no significant correlation between the TPC and TFC with antioxidant and enzymes inhibitory activities of *M. micrantha* extracts.
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