



**COMPUTATIONAL DESIGN AND DEVELOPMENT
OF MOLECULAR IMPRINT POLYMER FOR
SELECTIVE EXTRACTION OF
ANDROGRAPHOLIDE FROM *ANDROGRAPHIS
PANICULATA***

by

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TABLE OF CONTENTS

	PAGE
DECLARATION OF THESIS	i
ACKNOWLEDGEMENT	ii
TABLE OF CONTENTS	iii
LIST OF TABLES	viii
LIST OF FIGURES	ix
LIST OF ABBREVIATIONS	xi
LIST OF SYMBOLS	xiii
ABSTRAK	xiv
ABSTRACT	xv
CHAPTER 1: INTRODUCTION	1
1.1 Overview	1
1.2 Problem Statement	5
1.3 Scope of the Research	6
1.3.1 Overall Objective	6
1.3.2 Specific Objectives	7
CHAPTER 2: LITERATURE REVIEW	8
2.1 Herbal Medicines	8
2.2 <i>Andrographis Paniculata</i>	8
2.2.1 Traditional Uses of <i>A. paniculata</i>	9
2.2.2 Latest Researches on the Benefits of <i>A. paniculata</i>	10
2.2.3 Bioactive Compounds of <i>A. paniculata</i>	10
2.3 Andrographolide	14

2.3.1	Medicinal Properties of Andrographolide	14
2.3.2	Isolation of Bioactive	15
2.3.3	Quality Control of Bioactive Compounds	16
2.4	Molecular Imprinting Technology	16
2.5	Fundamentals of the Imprinting Process	17
2.5.1	Choice of Template	18
2.5.2	Functional Monomers	18
2.5.3	Cross-linkers	21
2.5.4	Solvents / Porogen	23
2.5.5	Initiator	25
2.6	Theory of Molecular Imprinting	25
2.6.1	Pre-arrangement	27
2.6.1.1	Covalent Approach	27
2.6.1.2	Non-covalent Approach	28
2.6.1.3	Semi-covalent Approach	29
2.6.2	Polymerization	30
2.6.2.1	Bulk Polymerization	33
2.6.2.2	Suspension Polymerization	33
2.6.2.3	Precipitation Polymerization	34
2.6.2.4	In-situ Polymerization	35
2.6.2.5	Multistep Swelling Polymerization	35
2.6.3	Template Removal	36
2.7	Rational MIP Design	36
2.7.1	Interaction between Functional Monomers and Template	37
2.7.2	Computational Optimization	38
2.8	Advantages and Applications of MIP	40
2.8.1	MIP Application in Separation/Enrichment	41

2.8.2	MIP Application in Quartz Crystal Microbalance Sensor	42
2.9	MIP Application in Analytical Separation	43
2.9.1	Solid Phase Micro-Extraction	43
2.9.2	Adsorption Isotherm	44
2.9.2.1	Scatchard Plot	44
2.9.2.2	Langmuir Isotherm	45
2.9.2.3	Freundlich Isotherm	45
2.9.2.4	Langmuir-Freundlich Isotherm	46
2.10	MIP Application in Sensor Development	46
2.10.1	Biosensor	48
2.10.2	Quartz Crystal Microbalance	49
2.10.3	Basic Principles and Analytical Process of QCM	49
2.10.4	MIP-QCM Biosensor	50
2.10.5	Electrochemical MIP Biosensor	51
CHAPTER 3:	METHODOLOGY	52
3.1	Summary of Methodology	52
3.2	Chemicals	54
3.3	Computational Studies	54
3.3.1	Computer Simulation Workplace	55
3.3.2	Model Building	55
3.3.3	Geometrical Optimization	56
3.3.4	Energy Calculations	57
3.4	MIP Synthesis	57
3.4.1	Template Removal Efficiency	61
3.5	Characterization of MIP and NIP	61
3.5.1	Chemical and Physical Characterization of MIP and NIP	61

3.5.2	Stock Solution and Calibration Curve Preparation	62
3.5.3	Dynamic Adsorption Study and Imprinting Factor	62
3.6	Binding Isotherm Study using SPME	63
3.7	Evaluation of MIP and NIP using QCM	64
3.6.1	Preparation of the Crystals	64
3.6.2	Electrodeposition of MIP and NIP on QCM Surface	65
3.6.3	MIP-QCM Analysis	66
3.7	<i>A. paniculata</i> Plant Extract Preparation	67
CHAPTER 4: RESULTS AND DISCUSSION		69
4.1	Rational MIP Design by Computational Approach	69
4.1.1	Conjecture of the Active Sites for the Template and Functional Monomers	69
4.1.2	Molecular Modelling and Geometrical Optimization of Template and Functional Monomers	70
4.1.3	Selection of Functional Monomer	71
4.1.4	Optimization of Template-Functional Monomer Ratio	77
4.2	MIP Synthesis	79
4.2.1	Template Removal Efficiency	83
4.3	Chemical and Physical Characterization of MIP and NIP	84
4.3.1	Functional Group Analysis of MIP and NIP using FTIR Technique	85
4.3.2	Morphological Characterization of MIP and NIP using SEM	87
4.3.3	The Dynamic Adsorption Study and Imprinting Factor of MIP	90
4.4	Evaluation of MIP and NIP using SPME	92
4.4.1	Adsorption Isotherm Study	92
4.4.2	Andrographolide Recovery from <i>A. paniculata</i> Extract	96
4.5	MIP and NIP Characterization by QCM	97
4.5.1	Electrodeposition of MIP using Cyclic Voltammetry	97

4.5.2	Dynamic Adsorption Study of MIP and NIP Sensor	100
4.5.3	Binding Assay of MIP-QCM Sensor	104
4.5.4	Binding Isotherm of MIP-QCM Sensor	105
4.5.5	MIP-QCM Sensor Response towards <i>A. paniculata</i> Extract	106
CHAPTER 5: CONCLUSION		108
5.1	Conclusion	108
5.2	Future Recommendations	110
5.3	Commercialization	111
REFERENCES		112
APPENDICES		128
LIST OF PUBLICATIONS		165

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LIST OF TABLES

NO.		PAGE
Table 2.1:	Latest Researches on Pharmacological Effects of <i>A. paniculata</i>	12
Table 2.2:	Acidic, Basic and Neutral Functional Monomers	19
Table 2.3:	Common Cross-linkers in MIP Synthesis	22
Table 2.4:	Non-Covalent Interactions and Corresponding Energies	29
Table 2.5:	Common Polymerization Methods in MIP Synthesis and Its Comparisons	31
Table 4.1:	Charge Difference Before and After Simulation	73
Table 4.2:	Gibbs Free Energy Changes (ΔE) due to the Complex Formation between the Template and the Functional Monomers	77
Table 4.3:	A Small Number of Examples of Research using Precipitation Polymerization Imprinting Process	80
Table 4.4:	Functional Groups and its Components	86
Table 4.5:	Particle Size and Polydispersity of MIP and NIP	89
Table 4.6:	Distribution Coefficient and Imprinting Factor of MIP and NIP	92
Table 4.7:	Fitting Parameters of Adsorption Isotherms of MIP and NIP by Non-linear Regression	95
Table 4.8:	Adsorption of Andrographolide from Plant Extract using MIP-SPME	96
Table 4.9:	Frequency Changes and Thickness of MIP Film	100

LIST OF FIGURES

NO.		PAGE
Figure 1.1:	<i>Andrographis Paniculata</i> Plant (<i>Hempedu Bumi</i>)	2
Figure 2.1:	Active Compounds Extracted from the whole Parts of <i>A. paniculata</i>	11
Figure 2.2:	Molecular Structure of Andrographolide	14
Figure 2.3:	Interaction Effects of Solvent Molecules in MIP Process	24
Figure 2.4:	MIP Synthesis; Three Step Process	26
Figure 2.5:	Pre-arrangement of Template and Functional Monomer using Computational Approach	40
Figure 2.6:	MIP in Sensor Application	47
Figure 3.1:	Flowchart of the Overall Research Methodology	53
Figure 3.2:	Molecular Modelling Techniques using HyperChem 8.0.10	56
Figure 3.3:	Schematic Diagram of MIP Synthesis Process	59
Figure 3.4:	Flowchart of MIP Synthesis Process	60
Figure 3.5:	Template Removal and Rebinding Step using SPME	63
Figure 3.6:	Complete System of QCM200 Quartz Crystal Microbalance	65
Figure 3.7:	Complete Set-up for Electrodeposition	66
Figure 3.8:	MIP Biosensor Evaluation Method	67
Figure 3.9:	<i>A. paniculata</i> Plant Extraction Method using Soxhlet Extractor	68
Figure 4.1:	Assessment of Active Sites in Template and Functional Monomers	72
Figure 4.2:	Optimized Structures of Pre-polymerization Complex at 1:1 (template: functional monomer) Ratio	75
Figure 4.3:	ΔE of Template - Functional Monomer Complexes in Different Mole Ratios of Monomers	79
Figure 4.4:	Template to Functional Monomer Ratio Effect on MIP Synthesis	81

Figure 4.5:	Polymerization at Molecular Level for 1 mol of Andrographolide	82
Figure 4.6:	Template Removal Efficiency Check through UV-Vis Spectrophotometry	84
Figure 4.7:	FTIR Spectrum	86
Figure 4.8:	SEM Micrograph of MIP, NIP and Size Distribution Histogram	88
Figure 4.9:	Dynamic Curves for the Sorption of Andrographolide on the Polymers	90
Figure 4.10:	Schematic Illustration of SPME Process and SPME Set-up	93
Figure 4.11:	Adsorption Isotherm Non-Linear Fitting	95
Figure 4.12:	Schematic Diagram of MIP Film Deposition on Gold Surface Transducer	98
Figure 4.13:	MIP Deposition on Quartz Crystal by Cyclic Voltammetry	99
Figure 4.14:	NIP Deposition on Quartz Crystal by Cyclic Voltammetry	99
Figure 4.15:	MIP-QCM and NIP-QCM Sensor Responses for Andrographolide	101
Figure 4.16:	QCM Sensor Response on Various Template Concentrations	103
Figure 4.17:	Mass Load by MIP-QCM and NIP-QCM Sensor with Pure Andrographolide	103
Figure 4.18:	Frequency Shift on Both MIP-QCM and NIP-QCM Sensor	104
Figure 4.19:	Langmuir Isotherm of MIP-QCM Sensor	105
Figure 4.20:	Calibration Curve of MIP-QCM Sensor with Template Solution	106

LIST OF ABBREVIATIONS

3-TAA	3-thiophene Acetic Acid
<i>A. paniculata</i>	<i>Andrographis Paniculata</i>
AA	Acrylic Acid
AAM	Acrylamide
Abs	Absorbance
AIBN	Azobisisobutyronitrile
AMBER	Assisted Model Building with Energy Refinement
CE	Counter Electrode
CEC	Capillary Electrochromatography
COOH	Carboxylic Group
CPU	Central Processing Unit
CV	Cyclic Voltammetry
DVB	Divinylbenzene
EGDMA	Ethylene Glycol Dimethacrylate
FTIR	Fourier Transform Infrared Spectroscopy
FT-NIR	Fourier Transform near Infrared Spectroscopy
FPIA	Fluorescence Polarization Immunoassays
GB	Gigabytes
HEMA	Hydroxymethyl Methacrylate
HIV	Human Immunodeficiency Virus
HPLC	High Performance Liquid Chromatography
HPTLC	High Performance Thin Layer Chromatography
H ₂ SO ₄	Sulphuric Acid
H ₂ O ₂	Hydrogen Peroxide

LIST OF ABBREVIATIONS

IA	Itaconic Acid
IF	imprinting factor
KCl	Potassium Chloride
LC-MS/MS	Liquid Chromatography – Mass Spectrometry
LOD	Limit of Detection
LOQ	Limit of Quantification
MAA	Methacrylic acid
MIP	Molecularly Imprinted Polymers
NIP	Non Imprinted Polymers
NH ₂	Amine
PM3	Parametric Model Number 3
PVDF	Polyvinylidene Flouride
QCM	Quartz Crystal Microbalance
RAM	Random Access Memory
RE	Reference Electrode
RHF	Restricted Hartree-Fock
RMS	Root-Mean-Square
RPM	Rotation per Minute
SPME	Solid Phase Micro-Extraction
TRIM	Trimethylolpropane Trimethacrylate
UV-Vis	Ultra Violet Visible Spectrophotometry
WE	Working Electrode

LIST OF SYMBOLS

ΔE	Binding Energy
A	Piezo-electrically active area
\AA	Angstrom
C_i	Initial Concentration
C_e	Equilibrium Concentration
K_d	Distribution Coefficient
kPa	Kilo Pascal
MHz	Mega Hertz
μl	Micro Litre
Q	Adsorption Binding Capacity
Q_{max}	Maximum Binding Capacity
K	Isotherm Constant
Q_e	Adsorption Capacity at Equilibrium
Δf	Frequency Shift
$^{\circ}\text{C}$	Degree Celcius
v	Volume of Sample Solution
m	Mass
f_0	Fundamental Resonant Frequency
Δm	Surface Mass Loading
ρ_q	Density of Quartz
μ_q	Shear Modulus
h	hour

Rekabentuk Pengkomputeran dan Pembinaan Polymer Molekul Bercetak untuk Pengekstrakan Andrographolide dari Andrographis Paniculata

ABSTRAK

Kajian ini telah menyelesaikan penyelidikan menyeluruh mengenai reka bentuk pengkomputeran dan pembangunan polimer yang dicetak secara molekul untuk pengekstrakan andrographolide dari Hempedu Bumi (*A. paniculata*). Pencetakan Molekul Polimer (MIP) adalah salah satu pendekatan “mangga dan kunci” di mana MIP adalah mangga dan asid kafein adalah kunci yang sesuai dengan mangga MIP secara fizikal dan kimia. Hyperchem 8.0.10 digunakan untuk mensimulasikan dan menentukan monomer yang berfungsi dengan betul dan nisbah yang sesuai dengan templat untuk tahap kerumitan yang tinggi. MIP disediakan hasil dari pemendakan pempolimeran tidak kovalen. Andrographolide, asid methakrylik, dan etilena glikol dimetakrilat digunakan sebagai templat, monomer yang berfungsi dan penghubung silang, masing-masing. Polimer tidak dicetak (NIP) telah dibangunkan dengan cara yang sama sebagai kawalan. Molekul template dalam zarah polimer MIP telah diekstrak dengan menggunakan sentrifugasi dan pengekstrakan fasa pepejal untuk membentuk rongga kosong untuk templat. MIP dan NIP dicirikan oleh FTIR, spektrofotometri UV dan kajian kinetik. Kecekapan MIP yang dicetuskan oleh andrographolide telah dinilai dalam pemisahan dan aplikasi sensor. Untuk analisis pemisahan, kajian penjerapan isotherm dan penyerapan assay dikendalikan. Faktor pencetakan dan pengedaran rongga masing-masing adalah 2.26 dan 55.45 ml.g⁻¹. Penyerapan assay dianalisa oleh tiga jenis penjerapan isotherm yang tidak linear. MIP yang dibangunkan berlandaskan isotherm Langmuir-Freundlich dengan kapasiti mengikat maksimum 149.59 µg.g⁻¹ di mana kapasiti ikatan eksperimen dikira sebagai 167.86 µg.g⁻¹. MIP dengan pengekstrekan mikro pepejal telah digunakan untuk mengekstrak andrographolide dari *A. paniculata* dengan kadar pemulihan sebanyak 92.3%. LOD dan LOQ untuk MIC-SPME adalah 0.14 dan 0.466 µg.ml⁻¹, masing-masing. MIP telah diaplikasi dalam pembangunan sensor dengan Quartz Crystal Microbalance (QCM) 200 di mana MIP telah dielektrodeposit dengan menggunakan kitaran voltametri. Peralatan QCM 200 menghasilkan perubahan frekuensi yang sepadan dengan penjerapan terpilih andrographolide dari *A. Paniculata*. Laman mengikat maksimum pada sensor MIP-QCM dengan menggunakan isotherm Langmuir linear ialah 18.02 µg.cm⁻². Di samping itu, sensor MIP-QCM boleh digunakan dalam analisis sampel sebenar. Ia didapati bahawa 45.53% daripada andrographolide dikesan dalam 0.10 µg.ml⁻¹ ekstrak tumbuhan dengan LOD dan LOQ 1.206 ng.cm⁻² dan 4.020 ng.cm⁻², masing-masing. Ini adalah penyelidikan pertama menggunakan sensor QCM berasaskan MIP untuk kuantifikasi andrographolide daripada *A. Paniculata*. Projek ini menunjukkan bahawa polimer yang dicetuskan oleh andrographolide boleh digunakan dalam pemisahan analitik dan pembangunan sensor untuk pengesanan dan kuantiti andrographolide dari ekstrak tumbuhan *A. Paniculata*. Oleh itu, polimer molekul bercetak yang kebaruan disintesis oleh pempolimeran yang boleh digunakan dalam kajian pengekstrekan dan sensor.

Computational Design and Development of Molecular Imprint Polymer for Selective Extraction of Andrographolide from Andrographis Paniculata

ABSTRACT

This research study highlighted on computational design and molecular imprint polymer development for the extraction of andrographolide from *Andrographis paniculata* (*A. Paniculata*). Molecularly Imprinted Polymer (MIP) is one of the “Lock and Key” approach, where MIP is the lock and andrographolide is the key which fits the MIP lock both physically and chemically. Hyperchem 8.0.10 software was used to simulate and determine the suitable functional monomer and optimum template-functional monomer ratio for the best complexity among them. The MIPs were prepared by non-covalent precipitation polymerization. Andrographolide, methacrylic acid, and ethylene glycol dimethacrylate were used as template, functional monomer, and cross-linker, respectively. Non-imprinted polymer (NIP) was developed in the same manner as a control. The template molecules were removed from MIP polymer particles using methanol: acetic acid (1:1 v/v) solvent. MIP and NIP were characterized by Fourier-transform Infrared Spectroscopy, Scanning Electron Microscope and dynamic adsorption study. The efficiency of andrographolide imprinted MIP were evaluated in separation and sensor applications. For the separation analysis, rebinding assay and adsorption isotherm studies were conducted. The imprinting factor of MIP is 2.26 with cavities distribution of 55.45 ml.g⁻¹. The binding assay was analyzed by three types of non-linear adsorption isotherm. The developed MIP follows Langmuir-Freundlich isotherm with maximum binding capacity of 149.59 µg.g⁻¹ where the experimental binding capacity was calculated as 167.86 µg.g⁻¹. MIP-SPME was used to extract andrographolide from *A. paniculata* with 92.3 % of recovery. The LOD and LOQ of MIP-SPME is 0.14 and 0.466 µg.ml⁻¹, respectively. MIP was applied in sensor development using Quartz Crystal Microbalance (QCM) 200 where MIP was electrodeposited using cyclic voltammetry. QCM 200 device gives the frequency changes corresponding to the selective adsorption of andrographolide from *A. paniculata*. The maximum binding sites on the MIP-QCM sensor by applying linear Langmuir isotherm is 18.02 ng.cm⁻². In addition, MIP-QCM sensor could be used in real sample analysis. It was found that 45.53 % of andrographolide detected in 0.10 µg.ml⁻¹ of plant extract with LOD and LOQ of 1.206 ng.cm⁻² and 4.020 ng.cm⁻² respectively. This is the first research using MIP based QCM sensor for the quantification of andrographolide from *A. paniculata*. This project demonstrated that the andrographolide imprinted polymer can be applied both in analytical separation and sensor development for the detection and quantification of andrographolide from *A. paniculata* plant extract. Therefore, a novel molecular imprint polymer was synthesized by precipitation polymerization which could be applied in separation and sensor studies.

CHAPTER 1: INTRODUCTION

1.1 Overview

Andrographis paniculata (*A. paniculata*) is a popular herbal plant from Acanthaceae family (Kumar et al., 2014). It was used as traditional and ethnomedicine for centuries in Asia, America and African continents (Lee et al., 2010; Okhwarobo et al., 2014; Yang & Song 2014; Ji et al., 2015). *A. paniculata* is commonly called as king of bitter, kalmegh and also locally known as “*Hempedu Bumi*” or “pokok ceria” in Malaysia. The whole plant have been used to cure various type of sicknesses. Since it is a traditional medicinal plant, it has various claims of uses with and without literature supports. The plant extract was used as remedy for severe diseases such as leprosy, influenza, dysentery, dyspepsia, wounds, chronic fever, cough, diarrhoea, ulcers and malaria (Okhwarobo et al., 2014). During recent decades, *A. paniculata* has attracted significant attention of modern drug discoverers and herbal researches. Several reports revealing diverse therapeutic potentials of different types of *A. paniculata* extracts (Thakur et al., 2015). Figure 1.1 shows the *A. paniculata* plant and the structure of the most active bioactive compound, andrographolide.

Andrographolide is an active element and the major constituent of *A. paniculata* (Kurzawa et al., 2015). Lately, numerous bio-activities of andrographolide have been reported. For example, immune-stimulatory activity, anti-inflammatory effect, cytokine induction or deduction, a potential cancer remedial agent and T cell activation suppression (Preet et al., 2014).

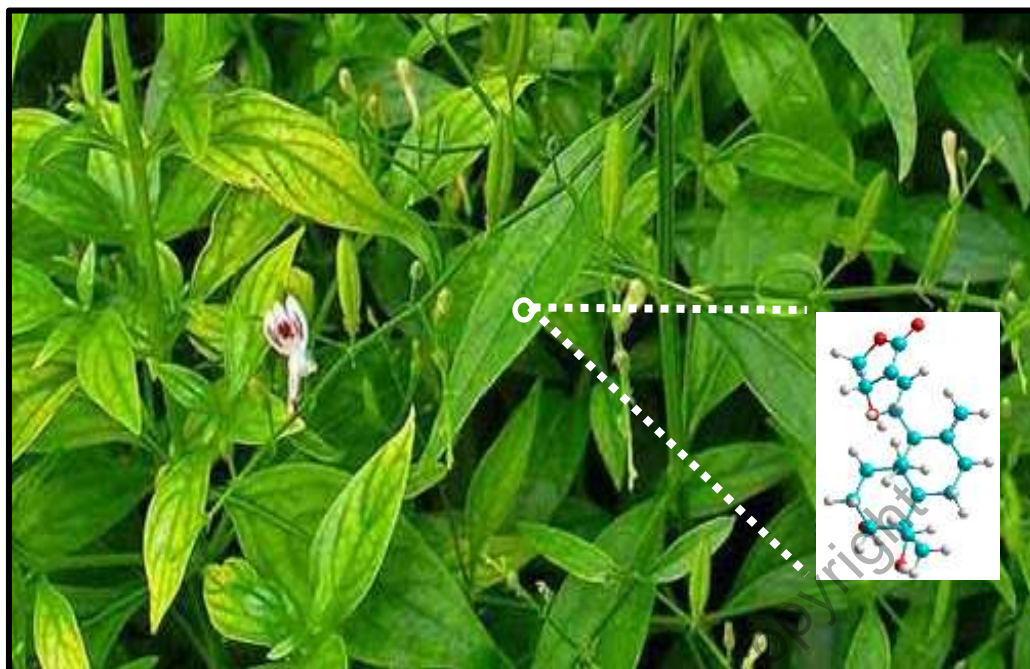


Figure 1.1: *Andrographis Paniculata* Plant (*Hempedu Bumi*)

Several researchers have reported that andrographolide have activity against a number of viruses, including HIV (Wang et al., 2011), hepatitis B (Chen et al., 2014), influenza (Seniya, Shrivastava, Singh, & Khan, 2014), hepatoma cancer cells (Ji et al., 2015) and hepatitis C (Hafid et al., 2017). It also has good curative effect on the upper respiratory tract infection and diarrhoea by bacterial and virus, which makes it as a natural antibiotics (Xiong et al., 2015). It is now accessible as commercial herbal medicine that acts as a potential cancer curing agent and other adverse diseases (Rajagopal et al., 2003).

Plants are always the key source of drug or treatment strategy in different traditional medicinal systems (Katiyar et al., 2012). Herbal products and their formulations have been prescribed for the mitigation and therapy of several diseases with human beings since a long time ago (Parasuraman et al., 2014). In recent years, many people prefer to choose plant based medicines or products to improve their health

conditions. It helps in treating certain types of sickness or diseases with no side effects or health consequences to human body. Although man made and artificial drugs are renowned in the market, herbal medicine consumptions are still ubiquitous currently due to their therapeutic applications (Ekor, 2014).

Standardized herbal extracts offer good prospects for new drugs development because of the unavailable similar compounds in chemical diversity. It is necessary to take proper actions to assure that potential active constituents are not lost, distorted or destroyed during isolation of bioactive compounds from plant samples to mimic as closely as possible the traditional herbal drug. The impurity of herbal products are always befalling when inaccuracy happens during the extraction process. The poor quality or impurity of herbal medicines can be attributed to adverse effects (Wang and Zhang, 2012). A suitable quality control required to check the authenticity, quality and purity of herbal products to utilize it to their full potential (Islam, Krishnan, Singh, & Ahmad, 2015). The extracted compounds from herbal plants needed a proper validation system to verify that they are pure and stable. Therefore, an analytical method is required for the extraction of these bioactive components.

Several methods have been suggested for the extraction of specific compounds from herbal plants. Recently, various promising methods were suggested for the extraction of specific compounds from herbal plants that can be used in pharmaceuticals and food industry, including immune-chromatography, fluorescence polarization immunoassays (FPIA) and molecularly imprinted polymers (Kwaśniewska et al., 2015). Conventional extraction methods have some limitations concerning the high solvent

consumption, the long extraction time required, and cost of expertise to operate the instruments.

Bioactive extraction became easier by modern chromatographic techniques however, the yield is dependent on the nature of materials to be studied. In solid-liquid extraction technique, the solvent, temperature, pressure and time are factors that affect the extraction methods. Owing to these reasons, it is important to redesign the conventional methods to a new technology that involves different conditions. The most noticeable recent technique is molecularly imprinted polymer. Molecular imprint polymer (MIP) is a rapidly emerging technology. It is a formation of artificial binding sites with a facsimile of shape and chemical-physical characteristics of the template molecule. This technique have been used to extract bioactive compounds from various plant materials (Saad, Madbouly, Ayoub, & El Nashar, 2015).

MIP synthesis is a broad technology which establishes detection properties into synthetic polymers (Piletsky et al., 2012). It is made of macromolecular materials that have the ability to selectively bind to specific target compound of interest (Wu et al., 2005). It gives a pre-arranged polymeric nanostructured materials by forming specific cavities similar to the template morphology (Nicholls et al., 2011; Piletsky et al., 2012; Vasapollo et al., 2011). In this study, molecular imprinting technology was used to extract andrographolide from *A. paniculata* extract with the help of solid phase micro extraction. A detailed study was conducted on MIP synthesis, characterization and application on andrographolide extraction which will be discussed in the following chapters.

1.2 Problem Statement

Quality assessment and standardization of pharmaceutically prepared plant based drugs requires ideally high quality plant extracts. Therefore, a suitable technique needs to be developed to selectively detect andrographolide bioactive compound from *A. paniculata* extract. The upgraded extraction techniques was easier than conventional methods such as high performance liquid chromatography, gas chromatography - mass spectrometry and immune-sorbent. However, these conventional equipment demand trained personnel to operate and also relatively expensive. Surrounding properties, solvent, temperature, pressure, and time are factors that affect the extraction methods and their attainment is still based on these factors and the nature of bioactive. It is known that conventional extraction methods have some limitations including the high solvent consumption, the long extraction time which affects the quality of the extracts. Andrographolide extraction from *A. paniculata* sample is difficult since it naturally coexist with several structural analogues. So, an appropriate method needs to be developed to extract andrographolide from *A. paniculata*.

MIP technique emerged as new extraction methods with selective extraction capacity. It is synthesized in various forms such as porous microspheres, thin films, bulk monoliths and hydrogels depending on the applications. MIP application as sorbent for solid phase extraction or sensor requires spherical and monodispersed spherical particles. Several polymerization methods have been developed to prepare spherical and uniform MIP, such as emulsion polymerization (Yang et al., 2015), multi-step swelling polymerization (Nakamura, Masumoto, Kubo, Matsunaga, & Haginaka, 2017) and suspension polymerization (Yan & Row, 2006). But these methods associated with some

complications such as complex procedures, addition of special stabilizers and surfactants. MIP for extraction purpose requires high porosity in a similar size of polymers for easier analytical methods.

MIP has drawbacks that needs to be overcome to obtain good affinity. For example, the size of MIP, porosity, affinity and physical and chemical stability must be enhanced. To develop MIP following these parameters, a rational design is necessary to choose the functional monomers, solvent and optimization of ratio between template and functional monomers.

1.3 Scope of the Research

The scope of this research is to computationally design and develop molecular imprint polymer for selective extraction of andrographolide from *A. paniculata* plant extract. The study was conducted to selectively extract bioactive compound using simple and efficient technique. Therefore, molecular imprint technique was collaborated with solid phase micro extraction (SPME) and Quartz Crystal Microbalance (QCM) for the extraction and quantification of andrographolide from the *A. paniculata* plant extract.

1.3.1 Overall Objective

The main objective is to synthesize novel molecular imprint polymer to selectively extract andrographolide bioactive element from *A. paniculata*.

1.3.2 Specific Objectives

- (i) To optimize the best template-functional monomer complex ratio using HyperChem 8.0.10 software.
- (ii) To synthesize MIP by precipitation polymerization technique using non-covalent approach.
- (iii) To characterize MIP using Fourier-transform Infrared Spectroscopy, Scanning Electron Microscope and dynamic adsorption study.
- (iv) To evaluate MIP and NIP by solid phase micro extraction and quartz crystal microbalance
- (v) To determine efficiency of the andrographolide imprinted MIP using *A. paniculata* extracts

CHAPTER 2: LITERATURE REVIEW

2.1 Herbal Medicines

Regardless of amazing advances in latest technologies in science and innovation, we are unable to give quality healthcare to all. Therefore, traditional herbal medicines are getting significant attention worldwide. Conventional medications especially traditional herbal medicines considered as a major healthcare provider all over the world. A vast group of people relies on herbal based supplements for their essential healthcare (Sen & Chakraborty, 2017). In fact, traditional herbal medicines have been used to treat illness since a long time ago with minimal or no industrial processes.

Currently, herbal medicine researches play a major role in global health as the usage of herbal medicines has increased vastly over the past three decades. Although herbal product have shown favourable capability on healing therapy, many of them remain unverified in terms of uses and pureness. Because of this inadequate knowledge, it leads to consequences such as adverse response and side effects (Ekor, 2014). The World Health Organization have made a large investments on herbal medicine research worldwide to produce quality herbal based medicines (Tilburt & Kaptchuk, 2008).

2.2 *Andrographis Paniculata*

A. paniculata is widely grown in wastelands and grasslands of tropical area. This is a very robust plant and can survive and adapt itself in a variety of soil conditions. In Malaysia, it is called “**hempedu bumi**” means the “**bile of earth**” which indicates the