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

by

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LIST OF ABBREVIATIONS

3-TAA	3-thiophene Acetic Acid
A. paniculata	*Andrographis Paniculata*
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_2SO_4	Sulphuric Acid
H_2O_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

Δ E	Binding Energy
A	Piezeo-electrically active area
Å	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
°C	Degree Celcius
v	Volume of Sample Solution
m	Mass
$f_\circ$	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 sabagai 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 $\mu g.g^{-1}$ di mana kapasiti ikatan eksperimen dikira sebagai 167.86 $\mu g.g^{-1}$. MIP dengan pengekstrekan mikro pepejal telah digunakan untuk mengekstrak andrographolide dari *A. panciulata* dengan kadar pemulihan sebanyak 92.3%. LOD dan LOQ untuk MIC-SPME adalah 0.14 dan 0.466 $\mu g.ml^{-1}$,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. Panciulata.* Laman mengikat maksimum pada sensor MIP-QCM dengan menggunakan isotherm Langmuir linear ialah 18.02 $\mu g.cm^{-2}$. Di samping itu, sensor MIP-QCM boleh digunakan dalam analisis sampel sebenar. Ia didapati bahawa 45.53% daripada andrographolide dikesan dalam 0.10 $\mu g.ml^{-1}$ 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 kebaharuan 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 asorption 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 $\mu g.g^{-1}$ where the experimental binding capacity was calculated as 167.86 $\mu g.g^{-1}$. 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 $\mu g.ml^{-1}$, 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. panciulata*. 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 $\mu g.ml^{-1}$ 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 Acanthacea family (Kumar et al., 2014). It was used as traditional and ethnomedicine for centuries in Asia, America and African continents (Lee et al., 2010; Okhuarobo 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 (Okhuarobo 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).

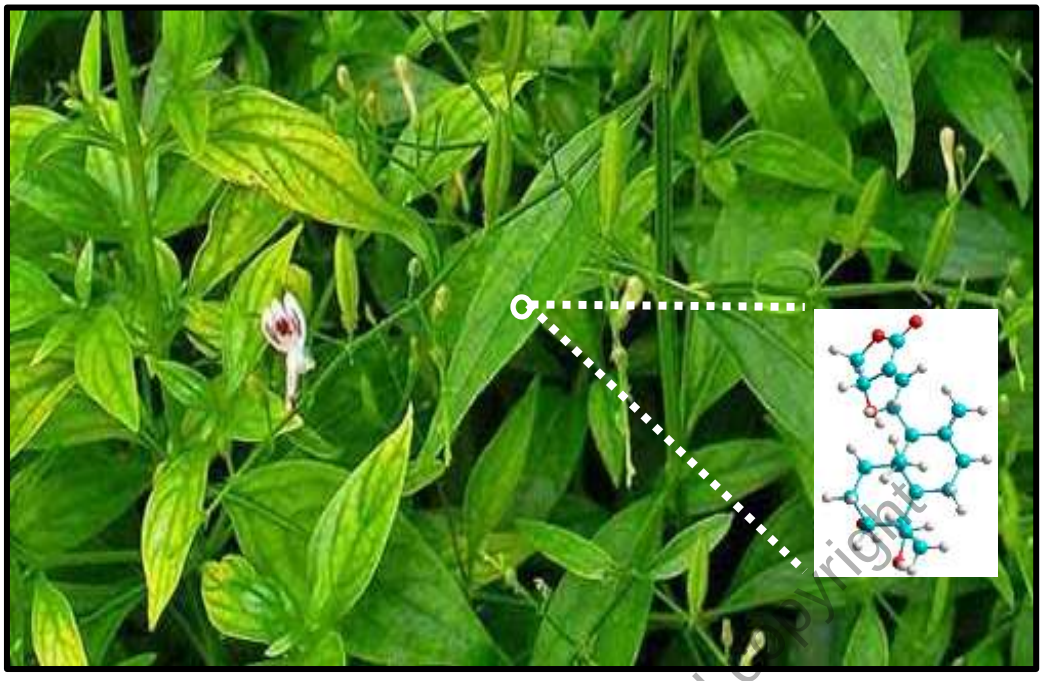

Figure 1.1: *Andrographis Paniculata* Plant (*Hempedu Bumi*)

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