UNIVERSITI PUTRA MALAYSIA

PRIORITIZATION OF NATURAL EXTRACTS BY LCMS-PCA IN IDENTIFICATION OF NEW PHOTOSENSITIZERS FOR PHOTODYNAMIC THERAPY

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By

NORAZWANA BINTI SAMAT

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

March 2015

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Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfillment of the requirement for the degree of Master of Science

PRIORITIZATION OF NATURAL EXTRACTS BY LCMS-PCA IN IDENTIFICATION OF NEW PHOTOSENSITIZERS FOR PHOTODYNAMIC THERAPY

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NORAZWANA BINTI SAMAT

March 2015

Chair: Khozirah binti Shaari, PhD

Faculty: Institute of Bioscience

Photodynamic therapy (PDT) is an alternative treatment for cancer that involves administration of a photosensitive drug or photosensitizer that localizes at the tumour tissue followed by in situ excitation at an appropriate wavelength of light. Tumour tissues are then killed by cytotoxic reactive oxygen species generated by the photosensitizer. Targeted excitation and photo-killing of affected tissues is achieved through focal light irradiation, thereby minimizing systemic side effects to the normal healthy tissues. Currently, there are only a small number of photosensitizers that are in the clinic and many of these share the same structural core based on cyclic tetrapyrroles. This study describes how metabolomic tools are utilized to prioritise natural extracts to search for structurally new photosensitizers from Malaysian biodiversity. As proof of concept, 278 photocytotoxic extracts has been analyzed using a hyphenated technique of liquid chromatography mass spectrometry coupled with principal component analysis (LCMS-PCA) and 27 extracts that potentially contained new photosensitizers were prioritized for chemical dereplication using an in-house UPLC-PDA-MS-Photocytotoxic assay platform. This has led to the identification of 2 new photosensitizers with cyclic tetrapyrrolic structures, thereby demonstrating the feasibility of the metabolomic approach.

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Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia
sebagai memenuhi keperluan untuk ijazah Sarjana Sains

PENGUTAMAAN EKSTRAK SEMULA JADI MENGGUNAKAN LCMS-PCA UNTUK MENGENAL PASTI SEBATIAN SENSITIF CAHAYA BAGI TERAPI FOTODINAMIK

Oleh

NORAZWANA BINTI SAMAT

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Terapi fotodinamik merupakan rawatan alternatif bagi merawat kanser. Terapi ini melibatkan pengenalan dadah yang sensitif terhadap cahaya yang akan terkumpul pada tisu tumor diikuti oleh pengujaan dadah pada gelombang cahaya yang sesuai. Tisu tumor kemudiannya dihapuskan oleh spesies oksigen reaktif yang toksik di mana ia dihasilkan oleh dadah sensitif cahaya. Pengujaan sasaran dan penghapusan tisu tumor oleh cahaya dicapai melalui sinaran cahaya tertumpu di mana kesan sampingan terhadap tisu normal dikurangkan. Setakat ini, hanya sebilangan kecil dadah sensitif cahaya yang digunakan secara klinikal dan kebanyakannya mempunyai struktur asas yang sama iaitu tetrapirola siklik. Kajian ini membincangkan penggunaan kaedah metabolomik ke atas ekstrak semula jadi untuk pengkhususan ekstrak yang mengandungi sebatian sensitif cahaya dengan struktur asas unik daripada biodiversiti di Malaysia. Sebagai bukti konsep, kami menganalisa 278 ekstrak toksik-cahaya menggunakan teknik gabungan kromatografi cecair bersama spektrometri jisim dan analisis komponen utama (LCMS-PCA) dan mengenal pasti 27 ekstrak yang berpotensi untuk mengandungi sebatian sensitif cahaya yang baru. Ekstrak-ekstrak yang berpotensi ini kemudiannya didireplikasi secara kimia menggunakan platform UPLC-PDA-MS- esei toksik cahaya. Kajian ini telah membuktikan kemampuan pendekatan metabolomik dengan penemuan 2 sebatian sensitif cahaya baru dengan struktur tetrapirola siklik.

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I certify that a Thesis Examination Committee has met on 10th March 2015 to conduct the final examination of Norazwana binti Samat on her thesis entitled "Prioritization of Natural Extracts by LCMS-PCA in Identification of New Photosensitizers for Photodynamic Therapy" in accordance with the Universities and University Colleges Act 1971 and the Constitution 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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LIST OF ABBREVIATIONS

ALA	Aminolevulinic acid
DMSO	Dimethyl sulfoxide
ESI	Electro spray ionization
FRIM	Forest Research Institute Malaysia
HDMS	High definition mass spectrometer
HPV	Human papilloma virus
HRMS	High resolution mass spectrometry
LCMS	Liquid chromatography mass spectrometry
MVA	Multivariate analysis
NMR	Nuclear magnetic resonance
PCA	Principal component analysis
PDA	Photodiode array
PDT	Photodynamic therapy
Q-TOF	Quadrupole, time-of-flight
RIA	Relative isotopic abundance
SBC	Sarawak Biodiversity Centre
SDTC	Sime Darby Technology Centre
UPLC	Ultra performance liquid chromatography
UV-vis	Ultra violet visible

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CHAPTER 1

INTRODUCTION

Photodynamic therapy (PDT) is a promising treatment for cancer which offers better selectivity, is less invasive and has no drug resistance compared to conventional chemotherapy (Bugaj 2011; Sharman et al. 1999; Wainwright 1996). Currently, the photosensitizers that are available in the clinics for cancer treatment are Photofrin®, aminolevulinic acid (ALA) and Foscan® and they have been applied for the treatment of cancers such as head and neck cancer, nodular basal cell carcinoma, and cholangiocarcinoma (van Oosten et al. 2006; Allison et al. 2005; Ortner 2004). Despite the advantages offered by PDT, it still suffers from several limitations and side effects such as burning sensation, pain and edema experienced by patient as well as ineffective penetration of light into treatment tissues (Ethirajan et al. 2011; Allison et al. 2005). Therefore, there are continual efforts to develop new clinically useful photosensitizers.

Chemical novelty obtainable from natural products is generally higher than that present in compounds from synthetic chemistry, making nature a very useful resource for new leads for drug development (Mishra, Tiwari 2011; Harvey 2000). Between years 1981 to 2010, about 60% of the anticancer drugs were inspired from natural products. Foscan is one example of photosensitizer drug that mimics a natural product molecule (Newman, Cragg 2012). Many of the early discovered drugs, such as vincristine and vinblastine isolated from *Catharanthus roseus* (plant), are still widely used to date. Despite this excellent track record, there is a general lack of systematic study of nature for new drugs. This is the case even for samples from the plant kingdom where most of the first natural drugs were discovered from, as only less than 15% of the higher plants have been systematically tested for biological activity (Bailly 2009). Conventional natural product drug discovery with exhaustive compound isolation is tedious, time-consuming and expensive (Tan et al. 2011a; Alali, Tawaha 2009; Cordell, Shin 1999). Furthermore, drug discovery efforts from nature, often times, lead to the rediscovery of known bioactive compounds.

There are however emerging efforts made to rapidly discover chemically unique compounds in bioactive extracts. Prathipati et al. for example, derived a Bayesian model using minimum inhibitory concentration data of 3779 compounds towards *Mycobacterium tuberculosis* H37Rv strain as a virtual screening tool to identify compounds which have potential as anti-tuberculosis agents (Prathipati et al. 2008). Hou et al. used the metabolic profile of microbial strains generated using liquid chromatography-mass spectrometry coupled with multivariate analysis for the purpose of discovering new drugs.

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They subjected 47 microbial strains to this untargeted LCMS-PCA method and were able to cluster microbial strains according to their secondary metabolites and then identified unique compounds that were present in one of the microbial strains (Hou et al. 2012). This report by Hou et al. is the first so far that utilizes metabolomics approach to prioritize samples in unbiased way to discover new chemical structures.

In this study, metabolomics approach based on PCA on LCMS profiles of plant extracts was used to rapidly prioritize the extracts that are more likely to contain new photosensitizers from a pool of bioactive extracts. This aspect of the work was a part of a screening program targeted at the identification of photocytotoxic natural extracts from a pool of more than 4000 samples from various collaborating research groups in Malaysia, with the goal of discovering novel chemical structures that may be developed into clinically useful photosensitizers. (Jong et al. 2013; Har et al. 2012; Tang et al. 2012; Kamarulzaman et al. 2011; Tan et al. 2011a; Tan et al. 2011b; Kamal et al. 2009; Ong et al. 2009; Chee et al. 2005). Photocytotoxic extracts were screened for their ability to reduce the percentage viability of HL60 promyelocytic leukemia cells by 50% or more when irradiated compared to a parallel experiment conducted in the dark. The preliminary screening succeeded in identifying several hundreds of bioactive extracts. From this huge number of extracts, an effective tool is required to fast-track the prioritization of extracts and identification of new and/or novel photosensitizing compounds.

This study began with the hypothesis that most of the plant extracts that are photocytotoxic would cluster together in PCA performed on their LCMS profiles because these extracts are likely to produce the same types of photosensitizers that are abundantly present and therefore already known. Thus, if and when a photocytotoxic extract is grouped separately from the common cluster of extracts, the extract would be more likely to contain new photosensitizers. Based on this, it is possible to prioritize the "outlier" samples for chemical dereplication by cross-referencing with an in-house photosensitizer database so that only the samples that did not contain known compounds were subjected to purification and identification of new photosensitizers. Thus, the objectives of this study are:

1. To optimize the method for LCMS-multivariate analysis (MVA) by using extracts spiked with known photosensitizers.

2. To identify plant extracts that potentially contain new photosensitizing compounds using the optimized method.

3. To isolate and identify the potentially new photosensitizers and to validate their bioactivity by measurement of their IC_{50} values in human promyelocytic leukemia cells (HL60).

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