UNIVERSITI PUTRA MALAYSIA

INFRARED-BASED METABOLOMICS FOR DISCRIMINATION OF Andrographis paniculata (Burm. F.) Wall. ex Nees ACCESSIONS HARVESTED AT DIFFERENT AGES AND TIMES

NUR ATHIFAH BINTI YUSOF

IB 2015 44

INFRARED-BASED METABOLOMICS FOR DISCRIMINATION OF *Andrographis paniculata* (Burm. F.) Wall. ex Nees ACCESSIONS HARVESTED AT DIFFERENT AGES AND TIMES

By

NUR ATHIFAH BINTI YUSOF

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

December 2015

COPYRIGHT

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

INFRARED-BASED METABOLOMICS FOR DISCRIMINATION OF *Andrographis paniculata* (Burm. F.) Wall. ex Nees ACCESSIONS HARVESTED AT DIFFERENT AGES AND TIMES

By

NUR ATHIFAH BINTI YUSOF

December 2015

Chairman : Associate Professor Intan Safinar Ismail, PhD
Institute : Bioscience

Andrographis paniculata is a herbal medicine for inflammation treatment over decades. The plant active metabolites such as andrographolide and neoandrographolide are mostly reported to have a wide range of medicinal properties. However, these two metabolites and also other metabolites could be affected by many factors. Harvesting age and time are among the factors which could influence the content of the compounds and efficacy of plant bioactivity. Hence, the purposes of this study were to identify the variation of major metabolites between three selected accessions which harvested at different harvesting age and time, and correlated to their nitric oxide (NO) inhibitory activity.

Metabolomics was utilized in detecting the differences in the metabolite profiles of these three accessions *A. paniculata*, 11265 (Perak, H), 11341 (Kelantan, P) and 11248 (Negeri Sembilan, T); which leaves were harvested at different ages and times. The discrimination of different harvesting ages and times were established by orthogonal partial least square-discriminant analysis (OPLS-DA) and characterized by Fourier Transform Infrared (FTIR) spectroscopy. Meanwhile, the harvesting age with the highest concentration of the major compounds particularly, andrographolide (A), and neoandrographolide (N) were further discriminated in order to compare between harvesting time of morning and evening. The partial least square (PLS) was applied to correlate the nitric oxide (NO) inhibitory and FTIR of the leaves harvested at different ages and times.

The OPLS-DA results revealed that morning harvested *A. paniculata* leaves at 120 days after transplanting (DAT) for P and T accessions, and 150 DAT for H accession contained the highest concentrations of the major compounds. These findings were further correlated to NO inhibitory activity in order to identify either these major compounds have influenced on this bioactivity. The methanol crude extracts of *A. paniculata* accessions showed the IC_{50} values ranged between 10.61±0.97 to 49.60±4.02 µg/mL for these three accessions. Some of the values are comparable to that of curcumin (IC_{50}, 14.69 µg/mL). The most potent samples in NO inhibition activity were represented by morning of 180 DAT for T and H accessions, and evening of 180 DAT for P accession. This study provides a standard procedure to harvest the *A. paniculata* leaves with highest potential of NO inhibitory activity. This spectroscopic-statistic approach could be useful as a quick guide for time and age of *A. paniculata* leaves should be harvested with targeted major compounds and highest potential for NO inhibitory activity.

Abstrak thesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains

METABOLOMIK BERASASKAN INFRAMERAH BAGI MEMBEZAKAN *Andrographis paniculata* (Burm. F.) Wall. ex Nees AKSESI YANG DITUAI PADA UMUR AND MASA YANG BERLAINAN

Oleh

NUR ATHIFAH BINTI YUSOF

Disember 2015

Pengerusi : Profesor Madya Intan Safinar Ismail, PhD
Institut : Biosains

Andrographis paniculata adalah ubatan herba bagi merawat keradangan sejak lebih berpuluh tahun. Aktif metabolik seperti andrografolid dan neoandrografolid sering dilaporkan mempunyai pelbagai ciri perubatan. Walaubagaimanapun, kandungan kedua-duanya dan juga metabolik lain boleh dipengaruhi oleh banyak faktor. Umur dan masa penuaian antara factor yang boleh mempengaruhi kandungan sebatian utama ini dan keberkesanan untuk bioaktivitinya. Oleh itu, tujuan kajian ini adalah untuk mengenalpasti perubahan metabolik utama bagi tiga aksesi yang dipilih untuk dituai pada umur dan masa penuaian yang berbeza dan kaitannya dengan perencatan aktiviti nitrik oksida.

Metabolomik digunakan untuk mengenalpasti perbezaan profil metabolik bagi tiga aksesi *A. paniculata*, 11265 (Perak, H), 11341 (Kelantan, P) dan 11248 (Negeri Sembilan, T) yang daunnya dituai pada umur dan masa yang berlainan. Pengkelasan bagi umur dan masa penuaian yang berbeza dibuat oleh analisa ortogon separa persegi diskriminan (OPLS-DA) dan penciriannya dilakukan oleh spektroskopi inframerah transformasi Fourier (FTIR). Manakala, umur penuaian yang mempunyai kandungan sebatian utama khususnya andrografolid dan neoandrografolid yang tinggi dikelaskan selanjutnya bagi membandingkan masa penuaian antara pagi dan petang. Analisa separa persegi (PLS) telah digunakan untuk mengaitkan perencatan aktiviti nitrik oksida (NO) dan FTIR apabila daun dituai pada umur dan masa yang berlainan.

Keputusan OPLS-DA menunjukkan daun *A. paniculata* yang dituai di waktu pagi pada umur 120 hari selepas dipindahkan untuk aksesi P dan T, dan 150 hari selepas dipindahkan untuk aksesi H mempunyai kandungan yang tinggi bagi kedua-dua

sebatian utama ini. Penemuan ini seterusnya dikaji untuk mengenalpasti samada perencatan aktiviti NO dipengaruhi oleh kandungan sebatian utama ini. Ekstrak metanol untuk aksesi *A. paniculata* menunjukkan nilai separuh kepekatan perencatan maksimum (IC_{50}) antara 10.61±0.97 hingga 49.60±4.02 µg/mL untuk ketiga-tiga aksesi. Sebahagian daripada nilai-nilai tersebut setanding dengan nilai curcumin (IC_{50}, 14.69 µg/mL). Sampel yang paling berpotensi untuk merencat aktiviti penghasilan nitrik oksida (NO) diwakili oleh daun yang dituai pada waktu pagi ketika berumur 180 hari selepas dipindahkn untuk aksesi T dan H, dan aksesi P diwakili oleh sampel yang dituai pada waktu petang ketika berumur 180 hari selepas dipindahkan. Kajian ini menyediakan prosedur yang standad untuk menuai daun *A. paniculata* yang berpotensi tinggi bagi merencatkan aktiviti nitrik oksida. Melalui pendekatan spektroskopi-statistik ini ia dapat digunakan sebagai panduan ringkas bagi menentukan masa dan umur penuaian untuk menuai daun *A. paniculata* dengan sebatian utama yang disasarkan dan berpotensi tinggi untuk merencatkan aktiviti nitrik oksida.

ACKNOWLEDGEMENTS

In the name of Allah, The Most Graceful and The Most Merciful. With all His blessings, finally this work has been successfully completed.

First and foremost, I offer my sincere gratitude to my supervisor, Assoc. Prof. Dr. Intan Safinar Ismail, for her excellent guidance, caring, patient and providing me with an excellent atmosphere for doing research from the first day I joined her research group. Thanks also for patiently correcting my writing and financially supported my research. She deserves appreciation and credit for what I have achieved today. Thank you, Dr.!

My appreciation also goes to Assoc. Prof. Dr. Alfi Khatib, Assoc. Prof. Dr. Yaya Rukayadi, and Dr. Daryush Talei for their valuable advice and suggestion. Credit also goes to Mrs. Rusnani Amirudin for ATR-FTIR spectroscopy, Mr. Tajuddin Abdul Manap for cultivation space of my plants, and Professor Johnson Stanslas for the provision of the pure diterpene lactones.

Many thanks to Mr. Azizul, a very helpful Research Officer who also has worked on *A. paniculata.* We have gone through a lot, from the day of the seed collection until the harvesting day. Thank you for his helping hands and continuous ideas in this project. I also would like to give my warm appreciation to Dr. Leong Sze Wei, who willing to help and teach me the cell work, starting from zero until I could do it on my own. I highly appreciated his time and patience in teaching me. My research would not have been possible to be completed without their helps.

Not forgetting to all my fellow friends especially; Ilya, my 24/7 source of positive vibes; Wani, my USM-UPM buddy; Ani, Amal, Yana, Raghu, Shikin, Kak Wan for sharing their metabolomics knowledge, for stimulating discussions and of course for all the fun we have had in the past few years.

I would also like to thank my parents, Mr. Yusof bin Salleh and Mrs. Mah Hasa binti Chik for supporting me spiritually and financially throughout my studies. Not to forget my elder sisters, Kak Long and Kak Ngah; and younger brothers, Abang and Adik. They were always supporting me and encouraging me with their best wishes.

Last but not least, my sincerest thanks to my beloved husband, Dr. Mohamad Hafizzudin, for his patience and love.

This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfillment of the requirement for the degree of Master of Science. The members of the Supervisory Committee were as follows:

Intan Safinar Ismail, PhD
Associate Professor
Institute of Biosciences
Universiti Putra Malaysia
(Chairman)

Yaya Rukayadi, PhD
Associate Professor
Faculty of Food Science and Technology
Universiti Putra Malaysia
(Member)

Nor Ainy Binti Mahyudin, PhD
Associate Professor
Faculty of Food Science and Technology
Universiti Putra Malaysia
(Member)

ROBIAH BINTI YUNUS, PhD
Professor and Dean
School of Graduate Studies
Universiti Putra Malaysia

Date:

Declaration by graduate student

I hereby confirm that:

- ¶ this thesis is my original work;
- ¶ quotations, illustrations and citations have been duly referenced;
- ¶ this thesis has not been submitted previously or concurrently for any other degree at any institutions;
- ¶ intellectual property from the thesis and copyright of thesis are fully-owned by Universiti Putra Malaysia, as according to the Universiti Putra Malaysia (Research) Rules 2012;
- ¶ written permission must be obtained from supervisor and the office of Deputy Vice-Chancellor (Research and innovation) before thesis is published (in the form of written, printed or in electronic form) including books, journals, modules, proceedings, popular writings, seminar papers, manuscripts, posters, reports, lecture notes, learning modules or any other materials as stated in the Universiti Putra Malaysia (Research) Rules 2012;
- ¶ there is no plagiarism or data falsification/fabrication in the thesis, and scholarly integrity is upheld as according to the Universiti Putra Malaysia (Graduate Studies) Rules 2003 (Revision 2012-2013) and the Universiti Putra Malaysia (Research) Rules 2012. The thesis has undergone plagiarism detection software

Signature: _____ Date: _____

Name and Matric No: Nur Athifah binti Yusof, GS31988

Declaration by Members of Supervisory Committee

This is to confirm that:

- ¶ the research conducted and the writing of this thesis was under our supervision;
- ¶ supervision responsibilities as stated in the Universiti Putra Malaysia (Graduate Studies) Rules 2003 (Revision 2012-2013) were adhered to.

Signature: _____
Name of Chairman of Supervisory Committee: Associate Professor Dr. Intan Safinar Ismail

Signature: _____
Name of Member of Supervisory Committee: Associate Professor Dr. Yaya Rukayadi

Signature: _____
Name of Member of Supervisory Committee: Associate Professor Dr. Nor Ainy Binti Mahyudin

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

A	Absorbance
ANOVA	Analysis of Variance
ASCII	American Standard Code for Information Interchange
ATR	Attenuated Total Reflectance
Chx	Chlorhexidine
Clo	Clove
COX	Cyclooxygenase
CV-ANOVA	Cross Validation-Analysis of Variance
DAT	Days after Transplanting
DDM	Disc Diffusion Method
DMEM	Dulbecco's Modified Eagle's Medium
EDA	Exploratory Data Analysis
eNOS	endothelial Nitric Oxide Synthase
FBS	Fetal Bovine Serum
HCA	Hierarchical Cluster Analysis
IC_{50}	Half maximal inhibitory concentration
IFN-γ	Interferon-γ
iNOS	inducible Nitric Oxide Synthase
KCCM	Korea Culture Centre of Microorganism
LA	Luria agar
LPS	Lipopolysaccharides
IFN-γ	Interferon-gamma

IL	Interleukin
MAPK	Mitogen-activated protein kinase
MHA	Mueller-Hinton agar
mRNA	messenger ribonucleic acid
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
NADPH	Nicotamide-adenine-dinucleotide phosphate
NF-κβ	Nuclear Factor-κβ
nNOS	neuronal Nitric Oxide Synthase
NSAID	Nonsteroidal anti-inflammatory drug
PARC	Pattern Recognition
PBS	Phosphate buffer saline
PenStrep	Penicillin Streptomycin
PGE_2	Prostaglandin E_2
Pen Strep	Penicillin Streptomycin
Q^2	Goodness of prediction
R^2	Goodness of fit
SDA	Sabouraud Dextrose agar
SD-IR	Second derivative infrared spectroscopy
sh	shoulder
SPSS	Statistical Package for the Social Sciences

CHAPTER 1

INTRODUCTION

1.1 General Introduction

In today's research scenario, the plant scientists are looking for valuable natural resources that can serve as drugs and health care products with less/no adverse side effects. More than half of the world's population predominately relied on plants for medicinal purposes (Farnsworth, 1994). The development of rapid and accurate approach in finding the active secondary metabolites from medicinal plants indirectly will lead to the improvement in pharmaceutical and nutraceutical research fields. To date, natural product resources have been recognized as the undeniably sources for pharmaceutical development and drug discovery area. Thousands of plants were identified to possess great potential for medicinal use. From the total of drugs prescribed worldwide, 25% of them are from plant origins (WHO, 1992). Thus, it is not an overexaggeration to say that nature has a store house of remedies to cure ailments for the human beings.

1.2 Traditional Claims on *Andrographis paniculata*

Andrographis paniculata (Burm. F.) Wall. ex. Nees is commonly known among traditional herbalists and practitioners. This traditional medicinal plant has been widely used in Ayurvedic (Indian), Thai and Chinese medicine systems against various ailments and diseases (Madav et al., 1995; Huang, 1994). It also has been ascribed to possess many medicinal properties such as antimicrobial, anti-inflammatory, antimalarial, antidiarrheal and anticancer. However, there are two interesting properties; antimicrobial and anti-inflammatory which have gained the attention for further investigation in this current study. The *A. paniculata* was proven to be effectively used in the treatment of infection and inflammation (Ji et al., 2005). Infection and inflammation are two terms which are different but often correlated to one another. Infection is caused by microorganism, and inflammation is a response of the organism to the pathogen. Inflammation is not an infection even when an infection could cause inflammation.

1.3 Fourier Transform Infra Red (FTIR)

Plants such as *A. paniculata* usually installed with numerous metabolites with a single plant being able to produce many compounds at any given time. However, these organic compounds contain some common functional groups such as hyrdroxyls, methyls or alkyls, aromatic ring and many others. This natural phenomena provides us a complete catalogue of different metabolites in different chemical species. Fourier Transform Infra Red (FTIR) is one of the analytical methods used to identify the

functional groups of the compounds. FTIR has been a common analytical instrument in identifying the functional groups of the chemical compounds and assisting in the elucidation of the compounds' structures. It provides the fingerprinting which relate to specific functional groups. The specified functional groups and bonding arrangements for each chemical compound make them to be unique (Wu et al., 2008). The unique characteristics enable to be used in classification and identification of different samples (De Luca et al., 2011) even through this metabolomics approach. Since natural products deal with hundreds of compounds, FTIR spectroscopy is a useful, simple and easy metabolic fingerprinting or footprinting tool which can analyze and measure our "catalog" of nature simultaneously. Hence, FTIR in combination with metabolomics are useful tools in standardizing the components which related to the quality of a herbal medicine such as *Andrographis paniculata*.

1.4 Metabolomics

Decades ago, phytochemist endured laborious and time-consuming works in order to isolate and identify the active component from the medicinal plant. It involved a long journey starting from chromatographic separation techniques, structure elucidation and determination of biological activity for targeted potential compounds. However, a single active compound is usually presence in a plant in a very small amount which sometimes can be scarcely identified. The synergistic effect of multiple compounds for the plant bioactivity was also difficult to be determined. The relevant pharmacological activities from the isolated compounds may not be due by a single compound, but more probably by a combination of compounds. This combination is also known as pharmacodynamic synergism or pharmacokinetic influence (Rates, 2001). By this mutual effect of many compounds, the curative effect might be enhanced to its maximum. Since the science field of metabolomics was born, many analytical platforms were introduced assisting the return of the natural products research to the forefront of the present drug discovery field. Among those analytical technologies being used to quantify all the metabolites in the cell (the metabolome) are FTIR, Gas Chromatography (GC)-Mass Spectroscopy (MS), Liquid Chromatography (LC)-MS, and Nuclear Magnetic Resonance (NMR).

These analytical instruments generate numerous amounts of massive data for plant metabolites. Multivariate data analysis (MVDA) is a powerful statistical technique which allows analysis of large number of data sets according to their biological variability. MVDA enables the translation and deciphering of the metabolites chemical data into a simpler form. FTIR also one of the reliable yet simple analytical techniques which can be employed to examine the complex biological samples such as the plant leaves (Xiaobo et al., 2010; Jing et al., 2010; Sankaran et al., 2010; Foca et al., 2009).

1.5 Problem Statements

Since ages, *A. paniculata* has been used to treat many health problems due to the presence of the active metabolites in this plant. Commonly the therapeutic activity of the medicinal plants, such as *A. paniculata,* is influenced by the concentration of those bioactive metabolites. The difference in metabolite concentration has a significant effect on the bioactivity efficacy of a plant. Among the factors that could give the differences in the metabolite content are the plant age and time of harvesting. Thus, any herbal products should undergo standardization of the active metabolites as to maintain the consistency of the concentration which in turn preserve the efficacy of the bioactivity. Therefore, in this present study, *A. paniculata* leaves were harvested in different harvesting ages and times in order to understand the changes of the metabolites related to a selected bioactivity.

Despite other studies being done on *A. paniculata,* none has been carried out on the determination of its metabolites changes due to the chosen two biotic factors of age and time of harvest. As Harapan (H) from Perak was selected as the best accession of *A. paniculata* based on the related cytotoxicity properties (Abdalla, 2005), it was used as the comparison to the other two accessions. FTIR was selected as the instrumental tool to facilitate this study as it is less expensive and can be made possible for the obtained data to be used by those with the access of only this instrument.

1.6 Research Objectives

This study focused on the discrimination of three *A. paniculata* accessions based on their different harvesting ages and times in relation to nitric oxide inhibitory activity through the use of FTIR-metabolomics approach.

Through this approach, the main aim of of this study are as listed below:

1. To identify the variations of metabolites based on FTIR data analysis between the three *A. paniculata* accessions harvested at different harvesting ages.
2. To determine the influence of the harvesting times (morning and evening) on the major metabolites of the three *A. paniculata* accessions.
3. To evaluate the nitric oxide inhibitory activity of *A. paniculata* accessions in relation to the different harvesting ages and times.

REFERENCES

Abdalla, J. A. 2005. *Genetic variation and anticancer activity of Ndrographis paniculata germplasm from Malaysia*, Master Thesis, Universiti Putra Malaysia.

Abdullah, F., Ling, S., Man, S., Tan, A., Tan, H., & Abdullah, Z. (2012). Characterization and identification of *Labisia pumila* by multi-steps infrared spectroscopy. *Vibrational Spectroscopy*, *62*, 200-206. doi:10.1016/j.vibspec.2012.06.004.

Ahmad, S., & Hashim, N. M. (2006). Menganalisis pola dan arah aliran hujan di Negeri Sembilan menggunakan kaedah GIS poligon Thiessen dan kontur Isoyet. *Geografia: Malaysian Journal of Society and Space*, *2*(1), 105-113.

Akbar, S. (2011). *Andrographis paniculata*: A review of pharmacological activities and clinical effects. *Alternative Medicine Review*, 11(1), 66-77.

Aktan, F. (2004). iNOS-mediated nitric oxide production and its regulation. *Life Sciences*, *75*(6), 639-653. doi:10.1016/j.lfs.2003.10.042.

Al-Amin, M. D., Islam, M. M., Siddiqi, M. M. A., Akter, S., Ahmed, S., Haque, M. M., ... & Chowdhury, A. S. (2012). Neoandrographolide isolated from leaves of *Adhatoda vasica* Nees. *Dhaka University Journal of Science*, *60*(1), 1-3.

Ali, K., Iqbal, M., Fortes, A., Pais, M., Korthout, H., Verpoorte, R., & Choi, Y. (2013). Red wines attenuate TNF-α production in human histiocytic lymphoma cell line: An NMR spectroscopy and chemometrics based study. *Food Chemistry*, *141*(3), 3124-3130. doi:10.1016/j.foodchem.2013.06.001.

Aniel Kumar, O., Mutyala Naidu, L., & Raja Rao, K. G. (2010). *In vitro* antibacterial activity in the extracts of *Andrographis paniculata* Burm. F. *International Journal of PharmTech Research*, *2*(2), 1383-1385.

Atik, E., Onlen, Y., Savas, L., & Doran, F. (2008). Inducible nitric oxide synthase and histopathological correlation in chronic viral hepatitis. *International Journal of Infectious Diseases*, *12*(1), 12-15. doi:10.1016/j.ijid.2007.03.010.

Azizan, K., Baharum, S., Ressom, H., & Noor, N. (2012). GC-MS analysis and PLS-DA validation of the trimethyl silyl-derivatization techniques. *American Journal of Applied Sciences*, *9*(7), 1124-1136. doi:10.3844/ajassp.2012.1124.1136.

Batkhuu, J., Hattori, K., Takano, F., Fushiya, S., Oshiman, K., & Fujimiya, Y. (2002). Suppression of NO production in activated macrophages *in vitro* and *ex vivo* by neoandrographolide isolated from *Andrographis paniculata*. *Biological & Pharmaceutical Bulletin*, *25*(9), 1169-1174. doi:10.1248/bpb.25.1169.

Beckles, D. M., & Roessner, U. (2012). Plant metabolomics: Application and opportunities for agriculture biotechnology. In Altman, A., & Hasegawa, P., *In "Plant biotechnology and agriculture: Prospects for the 21st century,* pp. 67-81. Amsterdam: Elsevier. doi:10.1016/B978-0-12-381466-1.00005-5.

Beyoğlu, D., & Idle, J. (2013). Metabolomics and its potential in drug development. *Biochemical Pharmacology*, *85*(1), 12-20. doi:10.1016/j.bcp.2012.08.013.

Berg, D., Zhang, J., Weinstock, J., Ismail, H., Earle, K., & Alila, H. et al. (2002). Rapid development of colitis in NSAID-treated IL-10–deficient mice. *Gastroenterology*, *123*(5), 1527-1542. doi:10.1053/gast.2002.1231527.

Bhan, M., Dhar, A., Khan, S., Lattoo, S., Gupta, K., & Choudhary, D. (2006). Screening and optimization of *Andrographis paniculata* (Burm. F.) Nees for total andrographolide content, yield and its components. *Scientia Horticulturae*, *107*(4), 386-391. doi:10.1016/j.scienta.2005.09.001.

Bjarnason, I., Hayllar, J., Mac Pherson, A. J., & Russell, A. S. (1993). Side effects of nonsteroidal anti-inflammatory drugs on the small and large intestine in humans. *Gastroenterology,* 104(6), 1832-1847.

Borooah, D. D., & Borua, P. K. (2011). Column chromatography for isolation of andrographolide from *in vitro* grown medicinal plant-*Andrographis paniculata* Wall. Ex Nees. *Int J Appl Biol Pharm Technol*, *2*(3), 571-574.

Bylesjö, M., Rantalainen, M., Cloarec, O., Nicholson, J., Holmes, E., & Trygg, J. (2006). OPLS discriminant analysis: combining the strengths of PLS-DA and SIMCA classification. *Journal of Chemometrics*, *20*(8-10), 341-351. doi:10.1002/cem.1006.

Calabrese, C., Berman, S., Babish, J., Ma, X., Shinto, L., & Dorr, M. et al. (2000). A phase I trial of andrographolide in HIV positive patients and normal volunteers. *Phytotherapy Research*, *14*(5), 333-338. doi:10.1002/1099-1573(200008)14:5<333::aid-ptr584>3.0.co;2-d.

Cellini, F., Chesson, A., Colquhoun, I., Constable, A., Davies, H., & Engel, K. et al. (2004). Unintended effects and their detection in genetically modified crops. *Food and Chemical Toxicology*, *42*(7), 1089-1125. doi:10.1016/j.fct.2004.02.003.

Chan, W., Taylor, D., Willis, C., Bodden, R., & Fehlhaber, H. (1971). The structure and stereochemistry of neoandrographolide, a diterpene glucoside from *Andrographis paniculata* Nees. *Tetrahedron*, *27*(21), 5081-5091. doi:10.1016/s0040-4020(01)90763-x.

Chandrasekaran, C., Gupta, A., & Agarwal, A. (2010). Effect of an extract of *Andrographis paniculata* leaves on inflammatory and allergic mediators *in vitro*. *Journal of Ethnopharmacology*, *129*(2), 203-207. doi:10.1016/j.jep.2010.03.007.

Chang, R., Ding, L., Gai-Qing, C., Qi-Choa, P., Ze-Lin, Z., & Smith, K. (1991). Dehydroandrographolide succinic acid monoester as an inhibitor against the Human Immunodeficiency Virus. *Experimental Biology and Medicine*, *197*(1), 59-66. doi:10.3181/00379727-197-43225.

Chao, W., & Lin, B. (2010). Isolation and identification of bioactive compounds in *Andrographis paniculata* (Chuanxinlian). *Chinese Medicine*, *5*(1), 17. doi:10.1186/1749-8546-5-17.

Chatterjee, S. K., & Raychaudhuri, S. P. (1992). Cultivation and quality improvement of medicinal plants in West Bengal. *In Recent Advances in Medicinal Aromatic and Spice Crops* 2: 397-407. New Delhi,India.

Chen, L., Qiu, F., Wei, H., Qu, G., & Yao, X. (2006). Nine new ent-Labdane diterpenoids from the aerial parts of *Andrographis paniculata*. *Helvetica Chimica Acta*, *89*(11), 2654-2664. doi:10.1002/hlca.200690237.

Chen, X., Wu, D., He, Y., & Liu, S. (2009). Nondestructive differentiation of Panax species using visible and shortwave near-infrared spectroscopy. *Food and Bioprocess Technology*, *4*(5), 753-761. doi:10.1007/s11947-009-0199-6.

Chippada, S. C., & Vangalapati, M. (2011). Antioxidant, an anti-inflammatory and anti arthritic activity of *Centella asiatica* extracts. *J Chem Bio Phy Sci*, 1(2), 260-269.

Chiou, W., Chen, C., & Lin, J. (2000). Mechanisms of suppression of inducible nitric oxide synthase (iNOS) expression in RAW264.7 cells by andrographolide. *British Journal of Pharmacology*, *129*(8), 1553-1560. doi:10.1038/sj.bjp.0703191.

Chiou, W., Lin, J., & Chen, C. (1998). Andrographolide suppresses the expression of inducible nitric oxide synthase in macrophage and restores the vasoconstriction in rat aorta treated with lipopolysaccharide. *British Journal of Pharmacology*, *125*(2), 327-334. doi:10.1038/sj.bjp.0702073.

Choudhury, B., Haque, S., & Poddar, M. (1987). *In vivo* and *in vitro* effects of Kalmegh (*Andrographis paniculata*) extract and andrographolide on hapatic microsomal drug metabolizing enzymes. *Planta Med*, *53*(02), 135-140. doi:10.1055/s-2006-962655.

Coon, J. T., & Ernst, E. (2004). *Andrographis paniculata* in the treatment of upper respiratory tract infections: a systematic review of safety and efficacy. *Planta Med*, 70(4), 293-298. doi:10.1055/s-2004-818938.

Connor, J., Manning, P., Settle, S., Moore, W., Jerome, G., & Webber, R. et al. (1995). Suppression of adjuvant-induced arthritis by selective inhibition of inducible nitric oxide synthase. *European Journal of Pharmacology*, *273*(1-2), 15-24. doi:10.1016/0014-2999(94)00672-t.

Cubero-Leon, E., Peñalver, R., & Maquet, A. (2014). Review on metabolomics for food authentication. *Food Research International*, *60*, 95-107. doi:10.1016/j.foodres.2013.11.041.

Dawson, V. L., Dawson, T. M., Bartley, D. A., Uhl, G. R., & Snyder, S. H. (1993). Mechanisms of nitric oxide-mediated neurotoxicity in primary brain cultures. *The Journal of Neuroscience*, *13*(6), 2651-2661.

Dechatiwongse na Aydhya, T., Jewvachdamrongkul, Y., & Charalittumrong, P. 1988. Quality Evaluation of Vegetables Drugs, pp. 61-65. Department of Medical Sciences, Bangkok.

de las Heras, B., Abad, M., Silván, A., Pascual, R., Bermejo, P., Rodríguez, B., & Villar, A. (2001). Effects of six diterpenes on macrophage eicosanoid biosynthesis. *Life Sciences*, *70*(3), 269-278. doi:10.1016/s0024-3205(01)01402-3.

de la Mata, P., Dominguez-Vidal, A., Bosque-Sendra, J., Ruiz-Medina, A., Cuadros-Rodríguez, L., & Ayora-Cañada, M. (2012). Olive oil assessment in edible oil blends by means of ATR-FTIR and chemometrics. *Food Control*, *23*(2), 449-455. doi:10.1016/j.foodcont.2011.08.013.

De Luca, M., Terouzi, W., Ioele, G., Kzaiber, F., Oussama, A., & Oliverio, F. et al. (2011). Derivative FTIR spectroscopy for cluster analysis and classification of Morocco olive oils. *Food Chemistry*, *124*(3), 1113-1118. doi:10.1016/j.foodchem.2010.07.010.

Dunn, W. B., & Ellis, D. I. (2005). Metabolomics: Current analytical platforms and methodologies. *Trends in Analytical Chemistry*, 24(4), 285-294. doi:10.1016/j.trac.2004.11.021.

Edwards, H., Munshi, T., & Page, K. (2007). Analytical discrimination between sources of ginseng using Raman spectroscopy. *Analytical and Bioanalytical Chemistry*, *389*(7-8), 2203-2215. doi:10.1007/s00216-007-1605-4.

Ellis, D., Brewster, V., Dunn, W., Allwood, J., Golovanov, A., & Goodacre, R. (2012). Fingerprinting food: current technologies for the detection of food adulteration and contamination. *Chem Soc Rev*, *41*(17), 5706. doi:10.1039/c2cs35138b.

Eriksson, L. (2006). *Multi- and megavariate data analysis*. Umeå, Sweden: Umetrics AB.

Farnsworth, N.R. (1994). *Ethnobotany and the search for new drugs*. New Jersey: J. Wiley.

Foca, G., Cocchi, M., Vigni, M., Caramanico, R., Corbellini, M., & Ulrici, A. (2009). Different feature selection strategies in the wavelet domain applied to NIR-based quality classification models of bread wheat flours. *Chemometrics and Intelligent Laboratory Systems*, *99*(2), 91-100. doi:10.1016/j.chemolab.2009.07.013.

Gallardo-Velázquez, T., Osorio-Revilla, G., Loa, M., & Rivera-Espinoza, Y. (2009). Application of FTIR-HATR spectroscopy and multivariate analysis to the quantification of adulterants in Mexican honeys. *Food Research International*, *42*(3), 313-318. doi:10.1016/j.foodres.2008.11.010.

George, M., & Pandalai, K. M. (1949). Investigations on plant antibiotics, Part IV. Further search for antibiotics substances in Indian medicinal plants. *Indian Journal of Medical Research*, 37, 169-181.

Girón, N., Pérez-Sacau, E., López-Fontal, R., Amaro-Luis, J., Hortelano, S., Estevez-Braun, A., & de las Heras, B. (2010). Evaluation of labdane derivatives as potential anti-inflammatory agents. *European Journal of Medicinal Chemistry*, *45*(7), 3155-3161. doi:10.1016/j.ejmech.2010.04.007.

Gomez, P., Pillinger, M., Attur, M., Marjanovic, N., Dave, M., & Park, J. et al. (2005). Resolution of inflammation: Prostaglandin E2 dissociates nuclear trafficking of individual NF-β subunits (p65, p50) in stimulated rheumatoid synovial fibroblasts. *The Journal of Immunology*, *175*(10), 6924-6930. doi:10.4049/jimmunol.175.10.6924.

Gómez-Romero, M., Segura-Carretero, A., & Fernández-Gutiérrez, A. (2010). Metabolite profiling and quantification of phenolic compounds in methanol extracts of tomato fruit. *Phytochemistry*, *71*(16), 1848-1864. doi:10.1016/j.phytochem.2010.08.002.

Goodacre, R., Broadhurst, D., Smilde, A., Kristal, B., Baker, J., & Beger, R. et al. (2007). Proposed minimum reporting standards for data analysis in metabolomics. *Metabolomics*, *3*(3), 231-241. doi:10.1007/s11306-007-0081-3.

Gyawali, R., & Ibrahim, S. (2014). Natural products as antimicrobial agents. *Food Control*, *46*, 412-429. doi:10.1016/j.foodcont.2014.05.047.

Hall, R. (2006). Plant metabolomics: from holistic hope, to hype, to hot topic. *New Phytologist*, *169*(3), 453-468. doi:10.1111/j.1469-8137.2005.01632.x.

Heinrich, M. (2010). Ethnopharmacology in the 21st century-grand challenges. *Front Pharmacol*, *1*. doi:10.3389/fphar.2010.00008.

Heo, S., Yoon, W., Kim, K., Ahn, G., Kang, S., & Kang, D. et al. (2010). Evaluation of anti-inflammatory effect of fucoxanthin isolated from brown algae in lipopolysaccharide-stimulated RAW264.7 macrophages. *Food and Chemical Toxicology*, *48*(8-9), 2045-2051. doi:10.1016/j.fct.2010.05.003.

Hong, Y., Chao, W., Chen, M., & Lin, B. (2009). Ethyl acetate extracts of alfalfa (*Medicago sativa* L.) sprouts inhibit lipopolysaccharide-induced inflammation *in vitro* and *in vivo*. *Journal of Biomedical Science*, *16*(1), 64. doi:10.1186/1423-0127-16-64.

Huang, T. K. (1994). *Andrographis paniculata* (Chuanxinlian). In *Handbook of Compositions and Pharmacology of Traditional Chinese Medicine, ed.* by T. K. Huang, pp. 1462-1467, China Medical and Technology Press, Beijing.

Iruretagoyena, M. (2004). Andrographolide interferes with T-Cell activation and reduces experimental autoimmune encephalomyelitis in the mouse. *Journal of Pharmacology and Experimental Therapeutics*, *312*(1), 366-372. doi:10.1124/jpet.104.072512.

Isha, A., Suyut, M., Yusof, N., & Ismail, I. (2013). Optimization of extraction method and qualitative FT-NMR analysis on *Andrographis paniculata* Leaves. *The Open Conference Proceedings Journal*, *4*(1), 133-133. doi:10.2174/22102892013040101333.

Jacob, S. J. P., & Shenbagaraman, S. 2011. Evaluation of antioxidant and antimicrobial activities of the selected green leafy vegetables. *International Journal of PharmTech Research*, 3(1), 148-152.

Jarukamjorn, K., & Nemoto, N. (2008). Pharmacological aspects of *Andrographis paniculata* on health and its major diterpenoid constituent andrographolide. *Journal of Health Science*, *54*(4), 370-381. doi:10.1248/jhs.54.370.

Javidnia, K., Parish, M., Karimi, S., & Hemmateenejad, B. (2013). Discrimination of edible oils and fats by combination of multivariate pattern recognition and FT-IR spectroscopy: A comparative study between different modeling methods. *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*, *104*, 175-181. doi:10.1016/j.saa.2012.11.067.

Ji, L., Wang, Z., Dong, F., Zhang, W., & Wang, Z. (2005). Andrograpanin, a compound isolated from anti-inflammatory traditional Chinese medicine *Andrographis paniculata*, enhances chemokine SDF-1α-induced leukocytes chemotaxis. *J Cell Biochem*, *95*(5), 970-978. doi:10.1002/jcb.20464.

Jing, M., Cai, W., & Shao, X. (2010). Multiblock partial least squares regression based on wavelet transform for quantitative analysis of near infrared spectra. *Chemometrics and Intelligent Laboratory Systems*, *100*(1), 22-27. doi:10.1016/j.chemolab.2009.09.006.

Jung, Y., Lee, J., Kwon, J., Lee, K., Ryu, D., & Hwang, G. (2010). Discrimination of the geographical origin of beef by 1H NMR-based metabolomics. *Journal of Agricultural and Food Chemistry*, *58*(19), 10458-10466. doi:10.1021/jf102194t.

Kane, J., Shears, L., Hierholzer, C., Ambs, S., Billiar, T., & Posner, M. (1997). Chronic hepatitis C virus infection in humans: Induction of hepatic nitric oxide synthase and proposed mechanisms for carcinogenesis. *Journal of Surgical Research*, *69*(2), 321-324. doi:10.1006/jsre.1997.5057.

Karaman, İ., Şahin, F., Güllüce, M., Öğütçü, H., Şengül, M., & Adıgüzel, A. (2003). Antimicrobial activity of aqueous and methanol extracts of *Juniperus oxycedrus* L. *Journal of Ethnopharmacology*, *85*(2-3), 231-235. doi:10.1016/s0378-8741(03)00006-0.

Katajamaa, M., & Orešič, M. (2007). Data processing for mass spectrometry-based metabolomics. *Journal of Chromatography A*, *1158*(1-2), 318-328. doi:10.1016/j.chroma.2007.04.021.

Khairudin, K., Sukiran, N., Goh, H., Baharum, S., & Noor, N. (2013). Direct discrimination of different plant populations and study on temperature effects by Fourier transform infrared spectroscopy. *Metabolomics*, *10*(2), 203-211. doi:10.1007/s11306-013-0570-5.

Khoo, G., Clausen, M., Pedersen, B., & Larsen, E. (2011). Bioactivity and total phenolic content of 34 sour cherry cultivars. *Journal of Food Composition and Analysis*, *24*(6), 772-776. doi:10.1016/j.jfca.2011.03.004.

Kim, J., Choi, J., Ku, K., Kang, D., Kim, J., Park, J., & Lee, C. (2011). A Correlation between antioxidant activity and metabolite release during the blanching of *Chrysanthemum coronarium* L. *Bioscience, Biotechnology and Biochemistry*, *75*(4), 674-680. doi:10.1271/bbb.100799.

Kim, H., & Verpoorte, R. (2010). Sample preparation for plant metabolomics. *Phytochemical Analysis*, *21*(1), 4-13. doi:10.1002/pca.1188.

Kim, S., Min, S., Kim, J., Park, S., Kim, T., & Liu, J. (2008). Rapid discrimination of commercial strawberry cultivars using Fourier transform infrared spectroscopy data combined by multivariate analysis. *Plant Biotechnology Reports*, *3*(1), 87-93. doi:10.1007/s11816-008-0078-z.

Kim, S., Ban, S., Chung, H., Cho, S., Chung, H., & Choi, P. et al. (2004). Taxonomic discrimination of flowering plants by multivariate analysis of Fourier transform infrared spectroscopy data. *Plant Cell Rep*, *23*(4), 246-250. doi:10.1007/s00299-004-0811-1.

Koteswara Rao, Y., Vimalamma, G., Venkata Rao, C., & Tzeng, Y. M. (2004). Flavanoids and andrographolides from *Andrographis paniculata*. *Phytochemistry*, 65(16), 2317-2321.

Ku, K., Huang, Y., Huang, Y., & Chiou, W. (2008). Miyabenol A inhibits LPS-induced NO production *via* IKK/IκB inactivation in RAW264.7 macrophages: Possible involvement of the p38 and PI3K pathways. *Journal of Agricultural and Food Chemistry*, *56*(19), 8911-8918. doi:10.1021/jf8019369.

Kuiper, H., Kok, E., & Engel, K. (2003). Exploitation of molecular profiling techniques for GM food safety assessment. *Current Opinion in Biotechnology, 14*(2), 238-243. doi:10.1016/s0958-1669(03)00021-1.

Kulyal, P., Tiwari, U. K., Shukla, A., & Gaur, A. K. (2010). Chemical constituents isolated from *Andrographis paniculata. Indian journal of Chemistry. Section B, Organic including medicinal*, *49*(3), 356-359.

Kumar, A., Dora, J., Singh, A., & Tripathi, R. (2012). A review on King of Bitter (Kalmegh). *International Journal of Research in Pharmacy and Chemistry,* 2(1), 116-124.

Kumar, B., Verma, S., & Singh, H. (2011). Effect of temperature on seed germination parameters in Kalmegh (*Andrographis paniculata* Wall. ex Nees.). *Industrial Crops and Products*, *34*(1), 1241-1244. doi:10.1016/j.indcrop.2011.04.008.

Kwon, Y., Ahn, M., Park, J., Liu, J., In, D., Min, B., & Kim, S. (2014). Discrimination of cultivation ages and cultivars of ginseng leaves using Fourier transform infrared spectroscopy combined with multivariate analysis. *Journal of Ginseng Research, 38*(1), 52-58. doi:10.1016/j.jgr.2013.11.006.

Lattoo, S., Dhar, R., Khan, S., Bamotra, S., Bhan, M., Dhar, A., & Gupta, K. (2007). Comparative analysis of genetic diversity using molecular and morphometric markers in *Andrographis paniculata* (Burm. F.) Nees. *Genet Resour Crop Evol*, *55*(1), 33-43. doi:10.1007/s10722-007-9212-y.

Lee, K., Ab. Aziz, F., Syahida, A., Abas, F., Shaari, K., Israf, D., & Lajis, N. (2009). Synthesis and biological evaluation of curcumin-like diarylpentanoid analogues for anti-inflammatory, antioxidant and anti-tyrosinase activities. *European Journal of Medicinal Chemistry*, *44*(8), 3195-3200. doi:10.1016/j.ejmech.2009.03.020.

Liu, D., Li, Y., Xu, H., Sun, S., & Wang, Z. (2008). Differentiation of the root of cultivated ginseng, mountain cultivated ginseng and mountain wild ginseng using FT-IR and two-dimensional correlation IR spectroscopy. *Journal of Molecular Structure*, *883-884*, 228-235. doi:10.1016/j.molstruc.2008.02.025.

Liu, H., Sun, S., Lv, G., & Chan, K. (2006). Study on *Angelica* and its different extracts by Fourier transform infrared spectroscopy and two-dimensional correlation IR spectroscopy. *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*, *64*(2), 321-326. doi:10.1016/j.saa.2005.07.026.

Liu, J., Wang, Z., & Ge, B. (2008). Andrograpanin, isolated from *Andrographis paniculata*, exhibits anti-inflammatory property in lipopolysaccharide-induced macrophage cells through down-regulating the p38 MAPKs signaling pathways. *International Immunopharmacology, 8*(7), 951-958. doi:10.1016/j.intimp.2007.12.014.

Liu, J., Wang, Z., & Ji, L. (2007). *In vivo* and *in vitro* anti-inflammatory activities of neoandrographolide. *The American Journal of Chinese Medicine, 35*(02), 317-328. doi:10.1142/s0192415x07004849.

Liu, J., Wang, Z., Ji, L., & Ge, B. (2006). Inhibitory effects of neoandrographolide on nitric oxide and prostaglandin E2 production in LPS-stimulated murine macrophage. *Mol Cell Biochem, 298*(1-2), 49-57. doi:10.1007/s11010-006-9349-6.

Liu, Y., Zhang, G., Sun, S., & Noda, I. (2010). Study on similar traditional Chinese medicines *Cornu Cervi Pantotrichum*, *Cornu Cervi* and *Cornu Cervi Degelatinatu*m by FT-IR and 2D-IR correlation spectroscopy. *Journal of Pharmaceutical and Biomedical Analysis, 52*(4), 631-635. doi:10.1016/j.jpba.2010.01.045.

Madav, S., Tripathi, H. C., Tandan, S. K., Dinesh, K., & Lal, J. (1998). Anti-6+ allergic activity of andrographolide. *Indian Journal of Pharmaceutical Science,* 60, 176-178.

Madav, S., Tripathi, H. C., Tandan, S. K.., & Mishra, S. K.. (1995). Analgesic, antipyretic and antiulcerogenic effects of andrographolide. *Indian J Pharm Sci,* 57(3), 121-125.

Majee, C., Gupta, B. K., Mazumdar, R., & Chakraborthy, G. S. (2011). HPLC method development and characterization of bio-active molecule isolated from *Andrographis paniculata*. *International Journal of PharmTech Research*, *3*(3), 1586-1592.

Majno, G., & Joris, I. (2004). Cells, tissues and diseases. Oxford University, Oxford, Britain.

Martel-Pelletier, J. (2003). Therapeutic role of dual inhibitors of 5-LOX and COX, selective and non-selective non-steroidal anti-inflammatory drugs. *Annals of the Rheumatic Diseases, 62*(6), 501-509. doi:10.1136/ard.62.6.501.

Matheis, G., Sherman, M. P., Buckberg, G. D., Haybron, D. M., Young, H. H., & Ignarro, L. J. (1992). Role of L-arginine-nitric oxide pathway in myocardial reoxygenation injury. *American Journal of Physiology-Heart and Circulatory Physiology*, *262*(2), H616-H620.

Matsuda, T., Kuroyanagi, M., Sugiyama, S., Umehara, K., Ueno, A., & Nishi, K. (1994). Cell differentiation-inducing diterpenes from *Andrographis paniculata* Nees. *Chem Pharm Bull*, *42*(6), 1216-1225. doi:10.1248/cpb.42.1216.

Miranda, K., Espey, M., & Wink, D. (2001). A rapid, simple spectrophotometric method for simultaneous detection of nitrate and nitrite. *Nitric Oxide*, *5*(1), 62-71. doi:10.1006/niox.2000.0319.

Mishra, S. K., Sangwan, N. S., & Sangwan, R. S. (2007). *Andrographis paniculata* (Kalmegh): A review. *Pharmacogn Rev*, 1, 283-298.

Mishra, P., Singh, R., Gupta, A., Chaturvedi, A., Pandey, R., Tiwari, S., & Mohapatra, T. (2013). Antibacterial activity of *Andrographis paniculata* (Burm. F.) Wall ex Nees leaves against clinical pathogens. *Journal of Pharmacy Research*, *7*(5), 459-462. doi:10.1016/j.jopr.2013.05.009.

Moco, S., Forshed, J., De Vos, R., Bino, R., & Vervoort, J. (2008). Intra- and inter-metabolite correlation spectroscopy of tomato metabolomics data obtained by liquid chromatography-mass spectrometry and nuclear magnetic resonance. *Metabolomics*, *4*(3), 202-215. doi:10.1007/s11306-008-0112-8.

Mukherjee, P. K. (2002). Analytical profiles of selected medicinal plants. Quality control of herbal drugs. An approach to evaluation of botanicals (1st edn), pp. 74. New Delhi: Business Horizons.

Mulukuri, N. S., Mondal, N. B., Prasad, M. R., Renuka, S., & Ramakrishna, K. (2011). Research article isolation of diterpenoid lactones from the leaves of *Andrographis paniculata* and its anticancer activity. *Int J Pharmacogn Phytochem*, *3*(3), 39-42.

Nathan, C., & Xie, Q. (1994). Nitric oxide synthases: Roles, tolls, and controls. *Cell*, *78*(6), 915-918. doi:10.1016/0092-8674(94)90266-6.

Newman, D., & Cragg, G. (2007). Natural products as sources of new drugs over the last 25 years. *J Nat Prod*, *70*(3), 461-477. doi:10.1021/np068054v.

Niranjan, A., Tewari, S. K., & Tehri, A. (2010). Biological activities of Kalmegh (*Andrographis paniculata* Nees) and its active principles- A review. *Indian Journal of Natural Products and Resource*, 1(2), 125-135.

Okhuarobo, A., Ehizogie Falodun, J., Erharuyi, O., Imieje, V., Falodun, A., & Langer, P. (2014). Harnessing the medicinal properties of *Andrographis paniculata* for diseases and beyond: a review of its phytochemistry and pharmacology. *Asian Pacific Journal of Tropical Disease*, *4*(3), 213-222. doi:10.1016/s2222-1808(14)60509-0.

Oelofse, A., Malherbe, S., Pretorius, I., & Du Toit, M. (2010). Preliminary evaluation of infrared spectroscopy for the differentiation of *Brettanomyces bruxellensis* strains isolated from red wines. *International Journal of Food Microbiology, 143*(3), 136-142. doi:10.1016/j.ijfoodmicro.2010.08.004.

Parichatikanond, W., Suthisisang, C., Dhepakson, P., & Herunsalee, A. (2010). Study of anti-inflammatory activities of the pure compounds from *Andrographis paniculata* (Burm.F.) Nees and their effects on gene expression. *International Immunopharmacology*, *10*(11), 1361-1373. doi:10.1016/j.intimp.2010.08.002.

Paradkar, M., Sivakesava, S., & Irudayaraj, J. (2003). Discrimination and classification of adulterants in maple syrup with the use of infrared spectroscopic techniques. *Journal of the Science of Food and Agriculture*, *83*(7), 714-721. doi:10.1002/jsfa.1332.

Parasher, R., Upadhyay, A., Khan, N. A., & Dwivedi, S. K. (2011). Biochemical estimation and quantitative determination of medicinally important andrographolide in *Andrographis paniculata* at different growth stages. *Electronic Journal of Environmental, Agriculture and Food Chemistry,* 10(7), 2479- 2486.

Pei, L., Sun, S., Guo, B., Huang, W., & Xiao, P. (2008). Fast quality control of Herba *Epimedii* by using Fourier transform infrared spectroscopy. *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*, *70*(2), 258-264. doi:10.1016/j.saa.2007.07.053.

Pholphana, N., Rangkadilok, N., Saehun, J., Ritruechai, S., & Satayavivad, J. (2013). Changes in the contents of four active diterpenoids at different growth stages in *Andrographis paniculata* (Burm. F.) Nees (Chuanxinlian). *Chinese Medicine*, *8*(1), 2. doi:10.1186/1749-8546-8-2.

Pholphana, N., Rangkadilok, N., Thongnest, S., Ruchirawat, S., Ruchirawat, M., & Satayavivad, J. (2004). Determination and variation of three active diterpenoids in *Andrographis paniculata* (Burm. F.) Nees. *Phytochemical Analysis*, *15*(6), 365-371. doi:10.1002/pca.789.

Podlešny, J., & Podlešna, A. (2011). Effect of rainfall amount and distribution on growth, development and yields of determinate and indeterminate cultivars of blue lupin. *Polish Journal of Agronomy*, 4, 16-22.

Puri, A., Saxena, R., Saxena, R., Saxena, K., Srivastava, V., & Tandon, J. (1993). Immunostimulant agents from *Andrographis paniculata*. *J Nat Prod*, *56*(7), 995-999. doi:10.1021/np50097a002.

Pramanick, S., Banerjee, S., Achari, B., Das, B., Sen,, A., & Mukhopadhyay, S. et al. (2006). Andropanolide and isoandrographolide, minor diterpenoids from *Andrographis paniculata*: Structure and X-ray crystallographic analysis ⊥. *J Nat Prod*, *69*(3), 403-405. doi:10.1021/np050211n.

Radha, R., Sermakkani, M., & Thangapandian, V. (2011). Evaluation of phytochemical and antimicrobial activity of *Andrographis paniculata* Nees (Acanthaceae) aerial parts. *International Journal of Pharmacy and Life Sciences*, 2(2), 562-567.

Rajani, M., Shrivastava, N., & Ravishankara, M. (2000). A rapid method for isolation of andrographolide from *Andrographis paniculata* Nees (Kalmegh). *Pharmaceutical Biology*, *38*(3), 204-209. doi:10.1076/1388-0209(200007)3831-sft204.

Rajpar, I., Khanif, Y. M,, Hassan, Z. U., Shah, A. N., Arshad, M., Galani, S. (2011). Growth, herb yield and phytochemical contents in a medicinal herb *Andrographis paniculata* under saline irrigation. *Journal of Medicinal Plant Research*, *5*(23), 5528-5533.

Rao, Y., Vimalamma, G., Rao, C., & Tzeng, Y. (2005). Flavonoids and andrographolides from *Andrographis paniculata*. *Cheminform*, *36*(10). doi:10.1002/chin.200510225.

Rates, S. (2001). Plants as source of drugs. *Toxicon*, *39*(5), 603-613. doi:10.1016/s0041-0101(00)00154-9.

Rees, D., Cellek, S., Palmer, R., & Moncada, S. (1990). Dexamethasone prevents the induction by endotoxin of a nitric oxide synthase and the associated effects on vascular tone: An insight into endotoxin shock. *Biochemical and Biophysical Research Communications*, *173*(2), 541-547. doi:10.1016/s0006-291x(05)80068-3.

Reich, G. (2005). Near-infrared spectroscopy and imaging: Basic principles and pharmaceutical applications. *Advanced Drug Delivery Reviews*, *57*(8), 1109-1143. doi:10.1016/j.addr.2005.01.020.

Reid, L., Woodcock, T., O'Donnell, C., Kelly, J., & Downey, G. (2005). Differentiation of apple juice samples on the basis of heat treatment and variety using chemometric analysis of MIR and NIR data. *Food Research International*, *38*(10), 1109-1115. doi:10.1016/j.foodres.2005.03.005.

Richard, A. M., Gold, L. S., & Nicklaus, M. C. (2006). Chemical structure indexing of toxicity data on the internet: moving toward a flat world. *Current Opinion in Drug Discovery and Development*, *9*(3), 314.

Sankaran, S., Ehsani, R., & Etxeberria, E. (2010). Mid-infrared spectroscopy for detection of Huanglongbing (greening) in citrus leaves. *Talanta*, *83*(2), 574-581. doi:10.1016/j.talanta.2010.10.008.

Saxena, S., Jain, D., Gupta, M., Bhakuni, R., Mishra, H., & Sharma, R. (2000). High-Performance Thin-Layer chromatographic analysis of hepatoprotective diterpenoids from *Andrographis paniculata*. *Phytochemical Analysis*, *11*(1), 34-36. doi:10.1002/(sici)1099-1565(200001/02)11:1<34::aid-pca487>3.0.co;2-v.

Schauer, N., & Fernie, A. (2006). Plant metabolomics: towards biological function and mechanism. *Trends in Plant Science*, *11*(10), 508-516. doi:10.1016/j.tplants.2006.08.007.

Schulz, H., & Baranska, M. (2007). Identification and quantification of valuable plant substances by IR and Raman spectroscopy. *Vibrational Spectroscopy*, *43*(1), 13-25. doi:10.1016/j.vibspec.2006.06.001.

See, D., Mason, S., & Roshan, R. (2002). Increased Tumor Necrosis Factor Alpha (TNF-α) and Natural Killer cell (NK) function using an integrative approach in late stage cancers. *Immunological Investigations*, *31*(2), 137-153. doi:10.1081/imm-120004804.

Sharma, A., Lal, K., & Handa, S. (1992). Standardization of the indian crude drug kalmegh by high pressure liquid chromatographic determination of andrographolide. *Phytochemical Analysis*, *3*(3), 129-131. doi:10.1002/pca.2800030308.

Sheeja, K., & Kuttan, G. (2008). Effect of *Andrographis paniculata* as an adjuvant in combined chemo-radio and whole body hyperthermia treatment - A preliminary study. *Immunopharmacology and Immunotoxicology*, *30*(1), 181-194. doi:10.1080/08923970701692916.

Sheeja, K., Guruvayoorappan, C., & Kuttan, G. (2007). Antiangiogenic activity of *Andrographis paniculata* extract and andrographolide. *International Immunopharmacology*, *7*(2), 211-221. doi:10.1016/j.intimp.2006.10.002.

Shen, Y., Chen, C., & Chiou, W. (2002). Andrographolide prevents oxygen radical production by human neutrophils: possible mechanism(s) involved in its anti-inflammatory effect. *British Journal of Pharmacology*, *135*(2), 399-406. doi:10.1038/sj.bjp.0704493.

Shin, E., Zhou, H., Guo, L., Kim, J., Lee, S., & Merfort, I. et al. (2008). Anti-inflammatory effects of glycyrol isolated from *Glycyrrhiza uralensis* in LPS-stimulated RAW264.7 macrophages. *International Immunopharmacology*, *8*(11), 1524-1532. doi:10.1016/j.intimp.2008.06.008.

Shuib, N., Shaari, K., Khatib, A., Maulidiani, Kneer, R., & Zareen, S. et al. (2011). Discrimination of young and mature leaves of *Melicope ptelefolia* using 1H NMR and multivariate data analysis. *Food Chemistry*, *126*(2), 640-645. doi:10.1016/j.foodchem.2010.10.043.

Shyur, L., & Yang, N. (2008). Metabolomics for phytomedicine research and drug development. *Current Opinion in Chemical Biology*, *12*(1), 66-71. doi:10.1016/j.cbpa.2008.01.032.

Singh, P. K., Hasan, T., Prasad, O., Sinha, L., Raj, K., & Misra, N. (2006). FT-IR spectra and vibrational spectroscopy of andrographolide. *Spectroscopy: An International Journal*, *20*(5), 275-283.

Singh, P., Hasan, T., & Misra, N. (2009). Vibrational dynamics of the diterpene-neoandrographolide. *E-Journal of Chemistry*, *6*(1), 183-188. doi:10.1155/2009/369569.

Singha, P., Roy, S., & Dey, S. (2003). Antimicrobial activity of *Andrographis paniculata*. *Fitoterapia*, *74*(7-8), 692-694. doi:10.1016/s0367-326x(03)00159-x.

Siripong, P., Kongkathip, B., Preechanukool, K., Picha, P., Tunsuwan, K., & Taylor, W. C. (1992). Cytotoxic diterpenoid constituents from *Andrographis paniculata* Nees leaves. *Sci Asia*, 18, 187-194.

Smith W. L., DeWitt, D. L., & Garavito, R. M. (2000). Cyclooxygenases: structural, cellular, and molecular biology. *Annu Rev Biochem*, 69, 145-182. doi: 10.1146/annurev.biochem.69.1.145.

Snyder, A., Sweeney, C., Rodriguez-Saona, L., & Giusti, M. (2014). Rapid authentication of concord juice concentration in a grape juice blend using Fourier-transform infrared spectroscopy and chemometric analysis. *Food Chemistry*, *147*, 295-301. doi:10.1016/j.foodchem.2013.09.140.

Son, H., Kim, K., van den Berg, F., Hwang, G., Park, W., Lee, C., & Hong, Y. (2008). 1H Nuclear Magnetic Resonance-based metabolomic characterization of wines by grape varieties and production areas. *Journal of Agricultural and Food Chemistry*, *56*(17), 8007-8016. doi:10.1021/jf801424u.

Sostres, C., Gargallo, C., Arroyo, M., & Lanas, A. (2010). Adverse effects of non-steroidal anti-inflammatory drugs (NSAIDs, aspirin and coxibs) on upper gastrointestinal tract. *Best Practice & Research Clinical Gastroenterology*, *24*(2), 121-132. doi:10.1016/j.bpg.2009.11.005.

Srivastava, A., Misra, H., Verma, R., & Gupta, M. (2004). Chemical fingerprinting of *Andrographis paniculata* using HPLC, HPTLC and densitometry. *Phytochemical Analysis*, *15*(5), 280-285. doi:10.1002/pca.779.

Stefanovic-Racic, M., Stadler, J., & Evans, C. (1993). Nitric oxide and arthritis. *Arthritis & Rheumatism*, *36*(8), 1036-1044. doi:10.1002/art.1780360803.

Stuehr, D. (1999). Mammalian nitric oxide synthases. *Biochimica Et Biophysica Acta (BBA) - Bioenergetics*, *1411*(2-3), 217-230. doi:10.1016/s0005-2728(99)00016-x.

Subramanian, R., Zaini Asmawi, M., & Sadikun, A. (2011). A bitter plant with a sweet future? A comprehensive review of an oriental medicinal plant: *Andrographis paniculata*. *Phytochemistry Reviews*, *11*(1), 39-75. doi:10.1007/s11101-011-9219-z.

Suwanbareerak, K., & Chaichantipyuth, C. 1991. Num- Lai- Pung- Porn, *Andrographis paniculata*. *J Med Pla Thailand Soc,* 7, 3-9.

Talei, D., Valdiani, A., Abdullah, M., & Hassan, S. (2012). A rapid and effective method for dormancy breakage and germination of King of Bitters (*Andrographis paniculata* Nees.) seeds. *Maydica, 57(2), 98-105.*

Tan, H., Ling, S., & Chuah, C. (2010). Multi-step infrared macro-fingerprinting on leaves of *Phyllagathis praetermissa* from different localities in Peninsular Malaysia. *Vibrational Spectroscopy*, *52*(1), 48-53. doi:10.1016/j.vibspec.2009.10.001.

Tepsorn, R. 2009. *Antimicrobial activity of Thai traditional medicinal plants extract incorporated alginate-tapioca starch based edible films against food related bacteria including foodborne pathogens,* PhD Thesis, University of Hohenheim.

Tipakorn, N. 2002. *Effects of Andrographis paniculata (Burm. F.) Nees on performance, mortality and coccidiosis in broiler chickens,* PhD Thesis, Institute of Animal Physiology and Animal Nutrition, George-August-Universität, Göttingen.

Tortora GJ, Funke BR, Case CL (2001). *Microbiology: An Introduction*, 7th edition Benjamin Cummings Publishing, San Francisco, USA. p. 88.

Tsatsanis, C., Androulidaki, A., Venihaki, M., & Margioris, A. (2006). Signalling networks regulating cyclooxygenase-2. *The International Journal of Biochemistry & Cell Biology*, *38*(10), 1654-1661. doi:10.1016/j.biocel.2006.03.021.

Valdiani, A., Kadir, M., Tan, S., Talei, D., Abdullah, M., & Nikzad, S. (2011). Nain-e Havandi *Andrographis paniculata* present yesterday, absent today: a plenary review on underutilized herb of Iran's pharmaceutical plants. *Molecular Biology Reports*, *39*(5), 5409-5424. doi:10.1007/s11033-011-1341-x.

Vane, J., Mitchell, J., Appleton, I., Tomlinson, A., Bishop-Bailey, D., Croxtall, J., & Willoughby, D. (1994). Inducible isoforms of cyclooxygenase and nitric-oxide synthase in inflammation. *Proceedings of The National Academy Of Sciences*, *91*(6), 2046-2050. doi:10.1073/pnas.91.6.2046.

Vaclavik, L., Lacina, O., Hajslova, J., & Zweigenbaum, J. (2011). The use of high performance liquid chromatography–quadrupole time-of-flight mass spectrometry coupled to advanced data mining and chemometric tools for discrimination and classification of red wines according to their variety. *Analytica Chimica Acta*, *685*(1), 45-51. doi:10.1016/j.aca.2010.11.018.

Whiteman, M., Spencer, J., Zhu, Y., Armstrong, J., & Schantz, J. (2006). Peroxynitrite-modified collagen-II induces p38/ERK and NF-κB-dependent synthesis of prostaglandin E2 and nitric oxide in chondrogenically differentiated mesenchymal progenitor cells. *Osteoarthritis and Cartilage*, *14*(5), 460-470. doi:10.1016/j.joca.2005.11.002.

WHO – World Health Organization. (1992). Quality control methods for medicinal plants material, Geneva

Woo, Y., Kim, H., & Chung, H. (1999). Classification of cultivation area of ginseng radix with NIR and Raman spectroscopy. *Analyst*, *124*(8), 1223-1226. doi:10.1039/a902396h.

Wu, Y., Sun, S., Zhao, J., Li, Y., & Zhou, Q. (2008). Rapid discrimination of extracts of Chinese propolis and poplar buds by FT-IR and 2D IR correlation spectroscopy. *Journal of Molecular Structure*, *883-884*, 48-54. doi:10.1016/j.molstruc.2007.12.009.

Wu, T., Chern, H., Damu, A., Kuo, P., Su, C., Lee, E., & Teng, C. (2008). Flavonoids and ent-labdane diterpenoids from *Andrographis paniculata* and their antiplatelet aggregatory and vasorelaxing effects. *Journal of Asian Natural Products Research*, *10*(1), 17-24. doi:10.1080/10286020701273627.

Wu, C. C., and Thiemermann, C. 1996. Biological control and inhibition of induction nitric oxide synthase. *Method Enzymol*, 268: 408-420.

Wyzgoski, F., Paudel, L., Rinaldi, P., Reese, R., Ozgen, M., & Tulio, A. et al. (2010). Modeling relationships among active components in black raspberry (*Rubus occidentalis* L.) fruit extracts using high-resolution 1H Nuclear Magnetic Resonance (NMR) spectroscopy and multivariate statistical analysis. *Journal of Agricultural and Food Chemistry*, *58*(6), 3407-3414. doi:10.1021/jf904401j.

Xiaobo, Z., Jiewen, Z., Holmes, M., Hanpin, M., Jiyong, S., Xiaopin, Y., & Yanxiao, L. (2010). Independent component analysis in information extraction from visible/near-infrared hyperspectral imaging data of cucumber leaves. *Chemometrics and Intelligent Laboratory Systems*, *104*(2), 265-270. doi:10.1016/j.chemolab.2010.08.019.

Xie, H., Jiang, J., Chen, Z., Shen, G., & Yu, R. (2006). Chemometric classification of traditional Chinese Medicines by their geographical origins using near-infrared reflectance spectra. *Analytical Sciences, 22*(8), 1111-1116. doi:10.2116/Analsci.22.1111.

Xu, C., Chou, G., & Wang, Z. (2010). A new diterpene from the leaves of *Andrographis paniculata* Nees. *Fitoterapia, 81*(6), 610-613. doi:10.1016/j.fitote.2010.03.003.

Yance, D. (2006). Targeting angiogenesis with integrative cancer therapies. *Integrative Cancer Therapies, 5*(1), 9-29. doi:10.1177/1534735405285562.

Yao, X., Wainberg, M., & Parniak, M. (1992). Mechanism of inhibition of HIV-1 infection *in vitro* by purified extract of *Prunella vulgaris*. *Virology, 187*(1), 56-62. doi:10.1016/0042-6822(92)90294-y.

Yusof, N., Isha, A., Ismail, I., Khatib, A., Shaari, K., Abas, F., & Rukayadi, Y. (2014). Infrared-metabolomics approach in detecting changes in *Andrographis paniculata* metabolites due to different harvesting ages and times. *Journal of The Science of Food and Agriculture*, n/a-n/a. doi:10.1002/jsfa.6987.

Zamora, R., Vodovotz, Y., and Billiar, T. R. 2000. Review article: Inducible nitric oxide synthase and inflammatory diseases. *Molecular Medicine* 6(5): 347-373.

Zhao, H. Y., and Fang, W. Y. 1991. Antithrombotic effects of *Andrographis paniculata* Nees in preventing myocardial infection. *Chin Med J (Engl)* 104: 770-775.

Zhang, X., & Tan, B. (2000). Antihyperglycaemic and anti-oxidant properties of *Andrographis paniculata* in normal and diabetic rats. *Clin Exp Pharmacol Physiol, 27*(5-6), 358-363. doi:10.1046/j.1440-1681.2000.03253.x.