UNIVERSITI PUTRA MALAYSIA

NUCLEAR MAGNETIC RESONANCE METABOLOMICS APPROACH IN CHEMICAL AND PROTECTIVE EVALUATIONS OF Orthosiphon stamineus BENTH. LEAF EXTRACTS ON CISPLATIN-INDUCED NEPHROTOXICITY

RAGHUNATH PARIYANI

IB 2016 23

NUCLEAR MAGNETIC RESONANCE METABOLOMICS APPROACH IN CHEMICAL AND PROTECTIVE EVALUATIONS OF *Orthosiphon stamineus* BENTH. LEAF EXTRACTS ON CISPLATIN-INDUCED NEPHROTOXICITY

By

RAGHUNATH PARIYANI

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

December 2016

COPYRIGHT

DEDICATION

This thesis is dedicated to my beloved parents

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfillment of the requirement for the Degree of Doctor of Philosophy

NUCLEAR MAGNETIC RESONANCE METABOLOMICS APPROACH IN CHEMICAL AND PROTECTIVE EVALUATIONS OF *Orthosiphon stamineus* BENTH. LEAF EXTRACTS ON CISPLATIN-INDUCED NEPHROTOXICITY

By

RAGHUNATH PARIYANI

December 2016

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

Orthosiphon stamineus (OS), locally known in Malaysia as 'Misai Kucing', is a herbaceous shrub belonging to the family Lamiaceae. Dried leaves of OS is gaining wide acceptance and marketed in the form of herbal tea, known as Java tea, owing to its traditional and scientific claims on various health benefits. OS has been a well-known renoprotective agent primarily due to its diuretic potential. This research investigated the effects of commonly employed drying methods of OS leaves on their chemical constituent profile, and *in vivo* biological properties of the protective role in cisplatin induced nephrotoxicity using rats, through Nuclear Magnetic Resonance (NMR) metabolomics approach. The NMR spectra of rat urine and the OS leaf extracts were analysed and correlated using multivariate data analysis techniques employing metabolomics platform.

The ^{1}H NMR metabolite profiling of aqueous extract of OS leaves resulted in the identification of 31 metabolites. The presence of biologically active secondary metabolites including phenylpropanoids such as caffeic acid, protocatechuic acid, chlorogenic acid, flavonoids such as luteolin and apigenin, gallic acid and orthosiphol derivatives were confirmed by J resolved NMR technique. The HPLC - MS/MS analysis further confirmed the presence of these secondary metabolites. Metabolite fingerprinting in combination with multivariate analysis has successfully differentiated the three differently dried (Freeze, microwave and shade) OS leaves and established that the levels of 15 metabolites were varied significantly between the samples. The shade drying method retained maximum secondary metabolites followed by the microwave, while freeze drying retained the least. Assessment of the main beneficial properties, such as antioxidant, total phenolic and flavonoid contents of any tea preparation, confirmed that all the differently dried Java tea leaves gave good antioxidant activity, with the shade dried leaves recorded the highest level with an IC50 of 48.09 μg/mL. The chemical constituents correlated to the high antioxidant activity of the shade dried leaves were extracted from a Partial Least Square regression

(PLS) model. In addition, the toxicity profile of the microwave dried OS leaves was investigated through acute oral toxicity test in Sprague Dawley (SD) rats of both sexes, whereby, the no-observed-adverse-effect level (NOAEL) of aqueous, 50% ethanolic and ethanolic extracts of the microwave dried OS was determined as 5000 mg/kg body weight/day. Thus, it is presumed that the microwave dried leaves are safe to be used as an oral health supplement.

Cisplatin is an anticancer drug, which induces nephrotoxicity in a long term use. Metabolomic analysis of the rats' urine revealed the involvement of a total of 17 biochemical markers from TCA cycle, carbohydrate, amino acid, and polyamine metabolic pathways in cisplatin nephrotoxicity. To the best of knowledge, 6 of the 17 involved metabolites are newly established in this study. In order to evaluate the protective efficacy of OS in cisplatin nephrotoxicity, shade and microwave dried OS extracts were administered at doses of 100, 200 and 400 mg/kg body weight to rats. The results suggested the dose independency of the extracts. Treatment with 50% aqueous ethanolic extract of shade dried OS leaves (OSFS) exhibited moderate ameliorative effect observed through a statistically significant reduction in the levels of 8 biomarkers. It was also revealed that the aqueous extract of the shade dried leaves (OSAS) exhibited slightly deteriorative activity via disturbance in the energy metabolism and gut microflora. The higher concentration of the secondary metabolites such as caffeic acid, chlorogenic acid, protocatechuic acid and orthosiphol in OSFS could be correlated to the ameliorative activity as revealed from a Principal Component Analysis (PCA) between OSAS and OSFS. A prediction model on nephroprotective effect of OS was constructed through PLS regression analysis.

Thus, the impact of different drying techniques on chemical constituents of OS leaves was established. The metabolomics approach has proved to be successful in shedding light to the even minute variations in the biological profiles of the low intensity metabolites involved in the renal toxicity caused by cisplatin. A global comprehensive view of the OS effect in cisplatin toxicity was successfully profiled and correlated.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk Ijazah Doktor Falsafah

PENILAIAN KIMIA DAN KESAN PERLINDUNGAN MELALUI PENDEKATAN NUKLEAR MAGNETIK RESONAN METABOLOMIK EKSTRAK DAUN *Orthosiphon stamineus* BENTH. TERHADAP NEFROTOKSIK CISPLATIN

Oleh

RAGHUNATH PARIYANI

Disember 2016

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

Orthosiphon stamineus (OS), dikenali di Malaysia sebagai 'Misai Kucing'. Ia merupakan sejenis pokok herba renek dalam keluarga Lamiaceae. Daun OS yang telah dikeringkan semakin diterima ramai lalu dipasarkan dalam bentuk teh herba, yang dikenali sebagai teh jawa. Ini terjadi atas kepelbagaian manfaat kesihatan yang diuar-uarkan melalui pendekatan tradisional dan saintifik. OS telah dikenali sebagai ejen perlindungan buah pinggang yang terkenal kerana potensi diuretiknya. Kajian mengenai kesan kaedah pengeringan yang telah biasa diamalkan turut dilakukan pada daun OS bagi menentukan profil konstituen kimianya serta kajian biologi turut dilakukan secara *in vivo* bagi memastikan peranan perlindungannya pada tikus aruhan cisplatin yang mengakibatkan kesan nefrotoksik. Ia dilakukan melalui pendekatan metabolomik Nuklear Magnetik Resonan (NMR). Spektrum NMR daripada sampel air kencing tikus dan estrak daun OS dianalisis dengan mengaitkan platform metabolomik menggunakan teknik analisis data multivariat.

Metabolit profil pada ^{1}H NMR ekstrak air daun OS telah mengenalpasti 31 jenis metabolit. Kehadiran metabolit biologi sekunder yang aktif termasuk phenyl-propanoids seperti asid caffeic, asid protocatechuic, asid chlorogenic, flavonoid seperti luteolin dan apigenin, asid Gallic dan derivatif orthosiphol telah disahkan melalui teknik “J resolve” NMR. Analisis lanjut HPLC - MS / MS mengesahkan kehadiran metabolit sekunder ini. Teknik pengkelasan metabolit ditambah dengan analisa multivariat telah berjaya membezakan tiga kaedah pengeringan (sejuk beku, ketuhar gelombang mikro dan bawah teduhan) daun OS dan telah membuktikan bahawa 15 metabolit telah mengalami perbezaan ketara antara sampel. Kaedah pengeringan dibawah teduhan berjaya mengekalkan kehadiran metabolit sekunder yang paling tinggi diikuti oleh pengeringan gelombang mikro, serta penyejuk bekuan menunjukkan nilai yang paling rendah. Penilaian terhadap manfaat utama seperti antioksida mendapati, jumlah fenolik dan kandungan flavonoid sesuatu penyediaan teh mengesahkan bahawa kesemua daun teh jawa yang berbeza kaedah

pengeringannya memberikan aktiviti antioksidan yang baik di mana daun yang dikeringkan dibawah teduhan telah merakamkan nilai IC50 yang paling tinggi pada 48.09 μg / mL. Selanjutnya analisa separa persegi (PLS) telah digunakan untuk mengenalpasti konstituen kimia yang bertanggungjawab terhadap aktiviti antioksida yang tinggi dalam daun OS yang dikeringkan di bawah teduhan ini. Di samping itu, profil toksik daun OS yang dikeringkan dengan menggunakan gelombang mikro telah dikaji dengan menjalankan ujian oral toksiti pada kedua-dua jantina tikus Sprague Dawley (SD). Keputusan menunjukkan tiada pemerhatian-taraf-kesan-buruk (NOAEL) bagi ekstrak air, 50% etanol dan etanol pada dos 5000 mg/kg berat badan/hari. Oleh itu, daun OS yang telah dikeringkan menggunakan gelombang mikro dianggap selamat untuk digunakan sebagai makanan tambahan kesihatan melalui oral.

Cisplatin adalah ubat anti-kanser yang boleh menyebabkan implikasi nefrotoksik jika digunakan dalam jangka masa panjang. Analisa metabolomik ke atas air kencing tikus mendedahkan sebanyak 17 penanda biokimia daripada kitaran TCA, karbohidrat, asid amino, dan laluan metabolik poliamina dalam aktiviti nefrotoksik yang disebabkan oleh cisplatin. Enam, daripada 17 metabolit merupakan metabolit terbaru yang telah dibuktikan dalam kajian ini. Untuk menilai keberkesanan perlindungan OS dalam menangani kesan nefrotoksik ini, daun OS yang diekstrak melalui pengeringan di bawah teduhan dan ketuhar gelombang mikro telah digunakan dan diberi pada beberapa dos rawatan iaitu 100, 200 dan 400 mg / kg berat badan tikus. Hasil kajian mendapati keberkesanan ekstak tidak bergantung kepada dos rawatan yang diberikan. Sementara itu, awatan dengan 50% ekstrak ethanol daripada daun OS yang dikeringkan di bawah teduhan (OSFS) menunjukkan kesan rawatan yang sederhana. Ini disebabkan oleh pemerhatian terhadap kadar penurunan yang signifikan pada 8 penandabio. Ia juga mendedahkan bahawa ekstrak air daripada daun OS yang dikeringkan di bawah teduhan (OSAS) hanya mempunyai sedikit penurunan nilai aktiviti melalui gangguan dalam metabolisme tenaga dan usus mikroflora. Metabolit sekunder yang lebih tinggi kepekatannya seperti asid caffeic, asid chlorogenic, asid protocatechuic dan orthosiphol dalam OSFS boleh dikaitkan dengan aktiviti membaik pulih seperti yang dinyatakan daripada Analisia Komponen Utama (PCA) antara OSAS dan OSFS. Satu model ramalan mengenai kesan nefroprotektif OS telah dibina melalui analisis regresi PLS.

Oleh itu, kajian ini telah membuktikan bahawa teknik pengeringan yang berbeza akan memberi kesan kepada konstituen kimia dalam daun OS. Pendekatan metabolomik juga telah berjaya memberi penjelasan terhadap perubahan kecil dalam profil biologi metabolit yang terlibat dalam masalah toksik buah pinggang yang disebabkan oleh cisplatin. Rumusannya, liputan yang menyeluruh terhadap kesan toksik cisplatin telah berjaya diprofilkan dan dihubungkaitkan.

ACKNOWLEDGEMENTS

"MY nature is love Him. And therefore I love. I do not pray for any-thing. I do not ask for anything. Let Him place me wherever He likes. I must love Him for love's sake"- Swami Vivekananda

First of all, I am deeply indebted and grateful to the Chairman of Supervisory Committee, Assoc. Prof. Dr. Intan Safinar Ismail for the expert guidance and timely helps provided in the work. I cannot thank you enough for being a strong pillar of unstinting support throughout this research journey, and will always be more than a mentor. Thank you very much for the trust, care and affection.

I am deeply obliged and thankful to have a caring and assertive supervisory committee. Prof. Dr. Mohd Roslan Sulaiman has always been supportive and encouraging in all the pharmacological experiments. His valuable suggestions and discussions have enlightened the ideas. I am deeply grateful to Dr. Hazilawati Hamza for her time and contribution in the histopathological and biochemical works. Assoc. Prof. Dr. Alfi Khatib has been instrumental in coaching the foundations for complex metabolomic analyses. Thank you very much for the knowledge you shared in our lengthy discussions.

I wish to express my sincere gratitude to Prof. Dr. Nordin Hj. Lajis, Prof. Dr. Khozirah Shaari and Assoc. Prof. Dr. Faridah Abas for their valuable support in the work.

The co-operation and support from all the technical and administrative staffs of Laboratory of Natural Products is deeply acknowledged. My sincere thanks to Mr. Salahuddin Mohd Rauf and Mr. Azizul Isha for providing a conducive work atmosphere in the NMR spectroscopy unit and Phytochemistry lab, where I spent most of the time during the course of this work.

I am thankful to Dr. Azira Mohamed (Genome Malaysia, Bangi), Ms. Ang May Yen and Ms. Maggie Yip (Shimadzu, Malaysia) for their technical helps during this study. My heartfelt gratitude to the UKM animal house staffs for their cooperation towards smooth conduction of pharmacological works.

The days at Laboratory of Natural Products were fantastic, even when the mind was an emotional cocktail. Thank you my dearest friends and labmates for being very supportive and making these days memorable for life. Thank you Ramesh Kumar, Amalina Azam, Norzaini Johari, Ahmed Mediani, Karthivashan, Nurathifah Yusof, Ilya Iryani Mahmod, Safwan Bushtaman and Azliana Abu Bakar.

I am blessed to have friends, who are always there for me at anytime, anywhere. It would not be courteous to say thanks to you, because you are in my thoughts every day, dear Mrs. & Mr. Sajesh. Thanks to Mrs. & Mr. Suresh, Habeeb, Jooshil, Rajesh Menon & Nair, Venki, Harikumar and Rasheed for the helping hands.

I am deeply indebted to Mr. Faizal and Mr. Venkatesan for their affection and making my days comfortable in Malaysia. The financial support provided by Universiti Putra Malaysia through NRGS is deeply acknowledged.

Last but not least, I cannot thank you my loving family members for being the source of my inner strength. My dear parents, Mr. Krishnanunni and Mrs. Santhakumari, you gave me this life, kindled the thirst for knowledge and opened up the doors for me to chase the dreams. I will strive to keep you as proud parents. My dear soulmate Shyama, your relentless support, prayers, love and motivation are my fuel. You keep me moving, thank you. Thank you my dear brothers, sister in laws, nieces, nephew and in laws for the prayers and well wishes. Without you all I would not have been here today. Thank You!

I certify that a Thesis Examination Committee has met on 28 December 2016 to conduct the final examination of Raghunath Pariyani on his thesis entitled "Nuclear Magnetic Resonance Metabolomics Approach in Chemical and Protective Evaluations of *Orthosiphon stamineus* Benth. Leaf Extracts on Cisplatin-Induced Nephrotoxicity" 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 Doctor of Philosophy.

Members of the Thesis Examination Committee were as follows:

Md Zuki bin Abu Bakar @ Zakaria, PhD
Professor
Institute of Bioscience
Universiti Putra Malaysia
(Chairman)

Md Nordin bin Hj. Lajis, PhD
Professor
Faculty of Science
Universiti Putra Malaysia
(Internal Examiner)

Johnson Stanslas, PhD
Professor
Faculty of Medicine and Health Science
Universiti Putra Malaysia
(Internal Examiner)

Geoffrey A. Cordell, PhD
Professor Emeritus
University of Illinois
United States
(External Examiner)

NOR AINI AB. SHUKOR, PhD
Professor and Deputy Dean
School of Graduate Studies
Universiti Putra Malaysia

Date: 28 February 2017

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

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

Mohd Roslan Sulaiman, PhD
Professor
Faculty of Medicine and Health Sciences
Universiti Putra Malaysia
(Member)

Hazilawati Hamza, PhD
Associate Professor
Faculty of Veterinary Medicine
Universiti Putra Malaysia
(Member)

Alfi Khatib, PhD
Associate Professor
Faculty of Pharmacy
International Islamic University 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: Raghunath Pariyani / GS31248

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: Professor Dr. Mohd Roslan Sulaiman

Signature: _____
Name of Member of Supervisory Committee: Associate Professor Dr. Hazilawati Hamza

Signature: _____
Name of Member of Supervisory Committee: Associate Professor Dr. Alfi Khatib

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVATIONS

°C	Degree centigrade
%	Percentage
α	Alpha
β	Beta
γ	Gamma
δ	Delta
µm	Micro meter
µg	Microgram
µL	Micro litre
ANOVA	Analysis of variance
BCAA	Branched chain amino acids
COSY	Homonuclear correlation spectroscopy
DOSY	Diffusion ordered-NMR spectroscopy
DPPH	1,1-diphenyl-2-picrylhydrazyl radical
GAE	Gallic acid equivalent
GFR	Glomerular filtration rate
HCA	Hierarchical cluster analysis
HMBC	Heteronuclear multiple bond coherence
HPLC	High performance liquid chromatography
JRES NMR	J resolved NMR spectroscopy
MS	Mass spectrometry
NKEA	National Key Economic Area
NMR	Nuclear magnetic resonance spectroscopy

NP	Natural product
OPLS	Orthogonal Partial Least Squares
OPLS-DA	Orthogonal Partial Least Squares - Discriminant Analysis
OS	*Orthosiphon stamineus*
OSAM	Aqueous extract of microwave dried OS
OSFM	50% aqueous ethanolic extract of microwave dried OS
OSAS	Aqueous extract of shade dried OS
OSFS	50% aqueous ethanolic extract of shade dried OS
PC	Principal components
PCA	Principal Component Analysis
PLS	Partial Least Squares
PLS-DA	Partial Least Squares - Discriminant Analysis
QE	Quercetin equivalent
ROS	Reactive oxygen species
SD	Sprague Dawley
SD	Standard deviation
SEA	South - East Asia
TFC	Total flavonoid content
TPC	Total phenolic content
TSP	Trimethylsilylpropionic acid-d4 sodium salt

CHAPTER 1

INTRODUCTION

1.1 Research background

Historically, plants have been the forerunners in the prevention and cure against a wide spectrum of diseases. Apart from their use in traditional system treatment, the abundant store of unique and diverse chemical compounds present in plants has served as a prominent source of lead molecules in the modern drug discovery process. This is evident from the fact that natural products (NP) and their derived compounds constitute a nearly 50% share of total new chemical entities approved in the span of the past 33 years, until December 2014 (Newman and Cragg, 2012; Newman and Cragg, 2016).

However, the progress made in drug discovery in terms of the number of novel drugs based on NP is not in proportion with the magnitude of ethno-pharmacological claims on various plants. The commonly adopted approaches in NP research such as bioassay guided isolation of active principles and high throughput screening often failed to elicit the optimum activity. This is primarily due to the fact that the bioactivity of a plant often resulted from a cumulative interaction of a large number of phytoconstituents, which deprives the isolated compound from exhibiting the activity as how a complex matrix of crude extract does (Cordell and Colvard, 2012; Yuliana et al., 2013) .

Recent developments in the field of systems biology such as metabolomics allow the evaluation of the biological activity of unfractionated complex extracts using proper bioassays (Robinette et al., 2011). Here, a broad range of analytical techniques such as nuclear magnetic resonance (NMR) spectroscopy, mass spectrometry (MS) and/or chromatography characterises the complex diverse metabolite classes present in the crude extract and an overall picture of metabolites correlating to the bioactivity could be derived using proper data mining methods. Thus, this holistic approach facilitates the identification of multiple active compounds from a single extract and their interaction either synergistically or antagonistically in *in vivo* systems.

Metabolomic fingerprinting has been applied to novel research areas such as pharmacological properties of medicinal plants, drug discovery via bioprospecting and quality control of herbal drugs. High-resolution NMR spectroscopy is a simple, powerful and fastest approach and has been used in the NP research to identify and correlate both primary and secondary plant metabolites with the elucidated bioactivity. The applications of NMR in metabolic profiling of plant extracts have been well reviewed in several recent articles (Kim et al., 2011; Schripsema, 2010).

Orthosiphon stamineus (OS) is a herbal remedy used traditionally in the cure of various system disorders, primarily that of kidney. It is locally known as *Misai Kucing*

in Malaysia. An aqueous infusion of dried leaves of OS, known as Java tea, is widely consumed by the people in South - East Asia (SEA) and Europe as a health adjuvant and general tonic (Ameer et al., 2012; Yuliana et al., 2009). The standardization of herbal products is of paramount importance in order to ensure consistent biological effects. So far the standardisation of OS was based on the quantification of certain marker compounds such as rosmarinic acid or sinensetin, and the practice was to generalize the observed biological activity to the variations of these selected markers. However, the metabolomic approach offers a platform to realistically correlate the responsible metabolites to the activity, and enables simultaneous standardization of several compounds present in the extract with minimal time and effort.

Variations in the metabolic profiles of NP might be due to multitude of factors including differences in species, pre- and post-harvesting methods, adulteration, and extraction among many others (Van der Kooy et al., 2009; Wang et al., 2009). The subsequent effects on bioactivity profile warrant proper monitoring of these metabolite changes in order to ensure their safe and effective usage. Drying technique deserves special attention as one of the most important variables in the preparation of Java tea (OS) leaves. A metabolomic analysis on the differently dried (shade, microwave and freeze) Java tea leaves thus would be helpful in fingerprinting the metabolites, which serves as the basis for standardisation and quality control tools.

The metabolomics platform has proved its usefulness in the field of toxicology as it derives a comprehensive picture of the effect of toxin in the body by the determination of global metabolome levels and their interrelations (Ramirez et al., 2013). OS has been known particularly for the beneficial effects on renal system owing to its diuretic and free radical scavenging activities (Arafat et al., 2008; Olah et al., 2003). However, to the best of our knowledge, the potential of OS in the protection of kidney from toxins, which is one of the most important sites of toxicity, has not yet been studied. A systematic exploration using metabolomics allows the simultaneous understanding of the complex mode of action of the toxin as well as the potential intervention of the OS. The toxico-metabolomics approach thus helps in understanding the mechanisms of toxicity, identify the biomarkers, and predict the bioactivity of the extract, thus, results in improvement of safety, to the shortening of the lead identification and a cost reduction (Robertson et al., 2011; Ulrich-Merzenich et al., 2009).

A holistic evaluation of long term perspective rational evidence-based herbal treatment could lead to the discovery and development of effective phytomedical intervention, taking into account the interaction of multiconstituents in synergism or antagonism, thus ensuring better safety and efficacy of the usage of herbs used in traditional system treatment.

In this research, it is hypothesized that different drying methods employed in the production of OS leaves affect the chemical profile and biological properties of OS. Identification of a proper drying method, which retains maximum beneficial chemical constituents, and safe, as well as efficient pharmacological activity is important to be ensured before its usage.

1.2 Aims and objectives

The work presented in this thesis aimed to investigate the effects of the drying methods exerted on the chemical and biological properties of OS leaves using NMR metabolomics approach. The metabolomics tool was employed to detect and discriminate the modulatory effects of OS on cisplatin nephrotoxicity. The general objective of this research was to evaluate the quality, safety, efficacy and consistency (QSEC) parameters of OS leaves with regard to different drying techniques employed.

These were achieved through the following set of specific objectives:

- To establish the metabolic fingerprint of shade, microwave and freeze dried OS leaf extracts, and to correlate their antioxidant activity with the overall bioactive compounds.
- To determine primary toxicity profile of microwave dried OS leaves.
- To identify the biomarkers and underlying metabolic pathways involved in cisplatin nephrotoxicity.
- To evaluate the modulatory effect of various OS extracts in cisplatin-induced nephrotoxic biomarkers and to develop a validated regression model, correlating the phytoconstituents to nephroprotective activity.

1.3 Outline of thesis

This thesis is presented in seven chapters. The general introduction is described in Chapter 1. Chapter 2 focusses on the comprehensive review of the literatures related to this research. Relevant literatures on pharmacological and phytochemical studies on OS, metabolomics and cisplatin nephrotoxicity are reviewed. Chapter 3 discusses the application of nuclear magnetic resonance (NMR) spectroscopy and chemometric methods in achieving the metabolic fingerprint of shade, microwave and freeze dried leaves. The correlation of antioxidant activity of the OS leaf to its phytoconstituents was established using Partial Least Square (PLS) regression analysis. Chapter 4 emphasizes on the preliminary phytochemical and toxicological studies on microwave dried OS leaves. A comparative evaluation of the microwave and shade dried OS leaves chemical constituent profile using liquid chromatography-mass spectrometry (LC-MS/MS) analysis and an acute oral toxicity test to assess the safety of microwave dried OS leaves are described here. Chapter 5 deals with the NMR spectroscopic profiling of the metabolites in cisplatin nephrotoxic and normal rats. The chemometric data analysis tools were used to identify the biomarkers and the underlying metabolic pathways involved in cisplatin nephrotoxicity. Chapter 6 discusses the NMR metabolomic analysis of rat urine in order to understand the metabolic perturbations induced by the OS intervention in cisplatin nephrotoxic biomarkers. A correlation model comprising of the nephroprotective activity with the phytoconstituents of OS was established using PLS. Finally, the overall conclusions are summarised in chapter 7, along with the future perspectives of the results obtained in this thesis.

REFERENCES

Abdelwahab, S.I., Mohan, S., Mohamed Elhassan, M., Al-Mekhlafi, N., Mariod, A.A., Abdul, A.B., Abdulla, M.A., Alkharfy, K.M., 2011. Antiapoptotic and Antioxidant Properties of Orthosiphon stamineus Benth (Cat's Whiskers): Intervention in the Bcl-2-Mediated Apoptotic Pathway. Evidence-Based Complementary and Alternative Medicine: eCAM 2011, 156765.

Abdullah, N.R., Ismail, Z., Ismail, Z., 2009. Acute toxicity of Orthosiphon stamineus Benth standardized extract in Sprague Dawley rats. Phytomedicine: International Journal of Phytotherapy and Phytopharmacology 16(2-3), 222-226.

Adam, Y., Somchit, M.N., Sulaiman, M.R., Nasaruddin, A.A., Zuraini, A., Bustamam, A.A., Zakaria, Z.A., 2009. Diuretic properties of Orthosiphon stamineus Benth. Journal of Ethnopharmacology 124(1), 154-158.

Ahamed, M.B., Aisha, A.F., Nassar, Z.D., Siddiqui, J.M., Ismail, Z., Omari, S.M., Parish, C.R., Majid, A.M., 2012. Cat's whiskers tea (Orthosiphon stamineus) extract inhibits growth of colon tumor in nude mice and angiogenesis in endothelial cells via suppressing VEGFR phosphorylation. Nutrition and Cancer 64(1), 89-99.

Ahmida, M.H., Abdel-Gayoum, A., El-Fakhri, M., 2001. Effect of spironolactone on cisplatin induced nephrotoxicity in rabbits. Human and Experimental Toxicology 20(9), 453-459.

Akowuah, G., Zhari, I., Norhayati, I., Sadikun, A., Khamsah, S., 2004. Sinensetin, eupatorin, 3′-hydroxy-5,6,7,4′-tetramethoxyflavone and rosmarinic acid contents and antioxidative effect of Orthosiphon stamineus from Malaysia. Food Chemistry 87(4), 559-566.

Akowuah, G.A., Ismail, Z., Ahmad, M., 2012. HPLC–TOF/MS profile and nitric oxide scavenging activity of Orthosiphon stamineus leaf extracts. Asian Pacific Journal of Tropical Biomedicine 2(3), S1436-S1439.

Akowuah, G.A., Ismail, Z., Norhayati, I., Sadikun, A., 2005. The effects of different extraction solvents of varying polarities on polyphenols of Orthosiphon stamineus and evaluation of the free radical-scavenging activity. Food Chemistry 93, 311-317.

Al-Harbi, M., Osman, A.M., Al-Gharably, N., Al-Bekairi, A., Al-Shabanah, O., Sabah, D., Raza, M., 1995. Effect of desferrioxamine on cisplatin-induced nephrotoxicity in normal rats. Chemotherapy 41(6), 448-454.

Ali, B., Al-Wabel, N., Mahmoud, O., Mousa, H., Hashad, M., 2005. Curcumin has a palliative action on gentamicin-induced nephrotoxicity in rats. Fundamental and Clinical Pharmacology 19(4), 473-477.

Ali, B.H., Al Moundhri, M.S., 2006. Agents ameliorating or augmenting the nephrotoxicity of cisplatin and other platinum compounds: a review of some recent research. Food and Chemical Toxicology 44(8), 1173-1183.

Ali, K., Iqbal, M., Yuliana, N.D., Lee, Y.-J., Park, S., Han, S., Lee, J.-W., Lee, H.-S., Verpoorte, R., Choi, Y.H., 2013. Identification of bioactive metabolites against adenosine A1 receptor using NMR-based metabolomics. Metabolomics 9(4), 778-785.

Ali, K., Maltese, F., Zyprian, E., Rex, M., Choi, Y.H., Verpoorte, R., 2009. NMR metabolic fingerprinting based identification of grapevine metabolites associated with downy mildew resistance. Journal of Agricultural and Food Chemistry 57(20), 9599-9606.

Al-Majed, A.A., Abd-Allah, A.R., Al-Rikabi, A.C., Al-Shabanah, O.A., Mostafa, A.M., 2003. Effect of oral administration of Arabic gum on cisplatin-induced nephrotoxicity in rats. Journal of Biochemical and Molecular Toxicology 17(3), 146-153.

Alonso, A., Marsal, S., Julià, A., 2015. Analytical methods in untargeted metabolomics: state of the art in 2015. Frontiers in Bioengineering and Biotechnology 3.

Alshawsh, M.A., Abdulla, M.A., Ismail, S., Amin, Z.A., 2011. Hepatoprotective Effects of Orthosiphon stamineus Extract on Thioacetamide-Induced Liver Cirrhosis in Rats. Evidence-Based Complementary and Alternative Medicine: eCAM 2011, 103039.

Ameer, O.Z., Salman, I.M., Asmawi, M.Z., Ibraheem, Z.O., Yam, M.F., 2012. Orthosiphon stamineus: traditional uses, phytochemistry, pharmacology, and toxicology. Journal of Medicinal Food 15(8), 678-690.

Analysis and Forecasts. 2013. Tea: The Future is Green and Herbal - Global Markets, Competitors and Opportunities - 2013-2018. (http://www.reportlinker.com/p01907817/Tea-The-Future-is-Green-and-Herbal---Global-Markets-Competitors-and-Opportunities---2013-2018-Analysis-and-Forecasts); accessed May 2016.

Antunes, L.M., Darin, J.D., Bianchi Nde, L., 2001. Effects of the antioxidants curcumin or selenium on cisplatin-induced nephrotoxicity and lipid peroxidation in rats. Pharmacological Research 43(2), 145-150.

Appenroth, D., Fröb, S., Kersten, L., Splinter, F.-K., Winnefeld, K., 1997. Protective effects of vitamin E and C on cisplatin nephrotoxicity in developing rats. Archives of Toxicology 71(11), 677-683.

Arafat, O.M., Tham, S.Y., Sadikun, A., Zhari, I., Haughton, P.J., Asmawi, M.Z., 2008. Studies on diuretic and hypouricemic effects of Orthosiphon stamineus methanol extracts in rats. Journal of Ethnopharmacology 118(3), 354-360.

Arany, I., Safirstein, R.L., 2003. Cisplatin nephrotoxicity. Seminars in Nephrology 23(5), 460-464.

Awale, S., Tezuka, Y., Banskota, A.H., Kouda, K., Tun, K.M., Kadota, S., 2001. Five Novel Highly Oxygenated Diterpenes of Orthosiphon stamineus from Myanmar. Journal ofNatural Products 64, 592-596.

Awale, S., Tezuka, Y., Banskota, A.H., Kouda, K., Tun, K.M., Kadota, S., 2002. Four highly oxygenated isopimarane-type diterpenes of Orthosiphon stamineus. Planta Medica 68(3), 286-288.

Awale, S., Tezuka, Y., Banskota, A.H., Shimoji, S., Taira, K., Kadota, S., 2002a. Norstaminane- and isopimarane- type diterpenes of Orthosiphon stamienus from Okinawa. Tetrahedron 58, 5503-5512.

Awale, S., Tezuka, Y., Adnyana, K., Kadota, S., 2003. Highly-oxygenated isopimarane-type diterpenes from Orthosiphon stamineus of Indonesia and their nitric oxide inhibitory activity. Chemical and Pharmaceutical Bulletin. 51(3), 268-275.

Badary, O.A., Abdel-Maksoud, S., Ahmed, W.A., Owieda, G.H., 2005. Naringenin attenuates cisplatin nephrotoxicity in rats. Life Sciences 76(18), 2125-2135.

Basheer, M.K.A., Majid, A.M.S.A., 2010. Medicinal potentials of Orthosiphon stamineus Benth. WebmedCentral Cancer (1), 1-12

Beckonert, O., Keun, H.C., Ebbels, T.M., Bundy, J., Holmes, E., Lindon, J.C. and Nicholson, J.K., 2007. Metabolic profiling, metabolomic and metabonomic procedures for NMR spectroscopy of urine, plasma, serum and tissue extracts. Nature protocols 2(11), 2692-2703.

Beger, R.D., Sun, J., Schnackenberg, L.K., 2010. Metabolomics approaches for discovering biomarkers of drug-induced hepatotoxicity and nephrotoxicity. Toxicology and Applied Pharmacology 243(2), 154-166.

Boudonck KJ., Mitchell MW., Német, L., Keresztes, L., Nyska, A., Shinar, D., Rosenstock, M., 2009 Discovery of metabolomics biomarkers for early detection of nephrotoxicity. Toxicologic Pathology 37(3):280-92 doi:10.1177/0192623309332992

Bro, R., and Smilde, A. K. 2014. Principal component analysis. Analytical Methods 6, 2812–2831

Brunetti, C., George, R.M., Tattini, M., Field, K., Davey, M.P., 2013. Metabolomics in plant environmental physiology. Journal of Experimental Botany, ert244.

Bylesjö, M., Rantalainen, M., Cloarec, O., Nicholson, J. K., Holmes, E., and Trygg, J. 2006. OPLS discriminant analysis: combining the strengths of PLS-DA and SIMCA classification. Journal of Chemometrics. 20, 341–351.

Cardoso-Taketa, A.T., Pereda-Miranda, R., Choi, Y.H., Verpoorte, R., Villarreal, M.L., 2008. Metabolic profiling of the Mexican anxiolytic and sedative plant Galphimia glauca using nuclear magnetic resonance spectroscopy and multivariate data analysis. Planta Medica 74(10), 1295-1301.

Chanda, S., Dave, R., Kaneria, M., Shukla, V., 2012. Acute oral toxicity of Polyalthia longifolia var. pendula leaf extract in Wistar albino rats. Pharmaceutical Biology 50(11), 1408-1415.

Chen, M.-F., Yang, C.-M., Su, C.-M., Hu, M.-L., 2014. Vitamin C protects against cisplatin-induced nephrotoxicity and damage without reducing its effectiveness in C57BL/6 mice xenografted with Lewis lung carcinoma. Nutrition and Cancer 66(7), 1085-1091.

Ching, J., Soh, W.L., Tan, C.H., Lee, J.F., Tan, J.Y.C., Yang, J., Yap, C.W., Koh, H.L., 2012. Identification of active compounds from medicinal plant extracts using gas chromatography-mass spectrometry and multivariate data analysis. Journal of Separation Science 35(1), 53-59.

Cicero, A.F., De Sando, V., Izzo, R., Vasta, A., Trimarco, A., Borghi, C., 2012. Effect of a combined nutraceutical containing Orthosiphon stamineus effect on blood pressure and metabolic syndrome components in hypertensive dyslipidaemic patients: a randomized clinical trial. Complementary Therapies in Clinical Practice 18(3), 190-194.

Coen, M., Holmes, E., Lindon, J.C., Nicholson, J.K., 2008. NMR-based metabolic profiling and metabonomic approaches to problems in molecular toxicology. Chemical Research in Toxicology 21(1), 9-27.

Committee on Herbal Medicinal Products., 2011. Assessment report on Orthosiphon stamineus Benth., folium. European Medicines Agency, pp. 8-9.

Cordell, G.A., Colvard, M.D., 2012. Natural products and traditional medicine: turning on a paradigm. Journal of Natural Products 75(3), 514-525.

Cornelison, T.L., Reed, E., 1993. Nephrotoxicity and hydration management for cisplatin, carboplatin, and ormaplatin. Gynecologic Oncology 50(2), 147-158.

Davis, J.W., Kramer, J.A., 2006. Genomic-based biomarkers of drug-induced nephrotoxicity. Expert Opinion on Drug Metabolism and Toxicology 2(1):95-101

Dickey, D.T., Wu, Y.J., Muldoon, L.L., Neuwelt, E.A., 2005. Protection against cisplatin-induced toxicities by N-acetylcysteine and sodium thiosulfate as assessed at the molecular, cellular, and in vivo levels. Journal of Pharmacology and Experimental Therapeutics 314(3), 1052-1058.

Dieterle, F., Ross, A., Schlotterbeck, G., Senn, H., 2006. Probabilistic quotient normalization as robust method to account for dilution of complex biological mixtures. Application in 1H NMR metabonomics. Analytical Chemistry 78, 4281-4290.

Dixon, R.A., and Paiva, N.L., 1995. Stress-induced phenylpropanoid metabolism. The Plant Cell 7(7), 1085-1097.

Doan, D.D., Nguyen, N., Doan, H., Nguyen, T., Phan, T., Van Dau, N., Grabe, M., Johansson, R., Lindgren, G., Stjernström, N., 1992. Studies on the individual and combined diuretic effects of four Vietnamese traditional herbal remedies (Zea mays, Imperata cylindrica, Plantago major and Orthosiphon stamineus). Journal of Ethnopharmacology 36, 225-231.

Dong, G., Wang, J., Guo, P., Wei, D., Yang, M., Kong, L., 2015. Toxicity assessment of Arisaematis Rhizoma in rats by a 1H NMR-based metabolomics approach. Molecular BioSystems 11(2), 407-417.

Erdlenbruch, B., Nier, M., Kern, W., Hiddemann, W., Pekrun, A., Lakomek, M. 2001. Pharmacokinetics of cisplatin and relation to nephrotoxicity in pediatric patients. European Journal of Clinical Pharmacology 57(5):393-402

Eriksson, L., Kettaneh-Wold, N., Trygg, J., Wikström, C., Wold, S., 2006. Multi-and megavariate data analysis: Part I: Basic Principles and Applications.

Fiehn, O., 2002. Metabolomics–the link between genotypes and phenotypes. Plant Molecular Biology 48(1-2), 155-171.

Fiehn, O., Robertson, D., Griffin, J., van der Werf, M., Nikolau, B., Morrison, N., Sumner, L.W., Goodacre, R., Hardy, N.W., Taylor, C., 2007. The metabolomics standards initiative (MSI). Metabolomics 3(3), 175-178.

Florea, A.-M., Büsselberg, D., 2011. Cisplatin as an anti-tumor drug: cellular mechanisms of activity, drug resistance and induced side effects. Cancers 3(1), 1351-1371.

Fonville, J. M., Richards, S. E., Barton, R. H., Boulange, C. L., Ebbels, T. M. D., Nicholson, J. K., Holmes, E., Dumas, M.E., 2010. The evolution of partial least squares models and related chemometric approaches in metabonomics and metabolic phenotyping. Journal of Chemometrics 24, 636–649.

Friesen, R.W., Novak, E.M., Hasman, D., Innis, S.M., 2007. Relationship of dimethylglycine, choline, and betaine with oxoproline in plasma of pregnant women and their newborn infants. The Journal of Nutrition 137(12):2641-2646

Gatley, S.J., Sherratt, H., 1977. The synthesis of hippurate from benzoate and glycine by rat liver mitochondria. Submitochondrial localization and kinetics. Biochemical Journal 166, 39-47.

Ghaffari, H., Venkataramana, M., Nayaka, S.C., Ghassam, B.J., Angaswamy, N., Shekar, S., Sampath Kumara, K.K., Prakash, H.S., 2013. Hepatoprotective action of Orthosiphon diffusus (Benth.) methanol active fraction through antioxidant mechanisms: an in vivo and in vitro evaluation. Journal of Ethnopharmacology 149(3), 737-744.

Ghiculescu, R.A., Kubler, P.A., 2006. Aminoglycoside-associated Fanconi syndrome. American Journal of Kidney Diseases 48(6):e89-e93

Gibson, D., 2009. The mechanism of action of platinum anticancer agents—what do we really know about it?. Dalton Transactions (48), 10681-10689.

Global Analysis Report. 2013. Organic Beverage Opportunities in the United States, Market Access Secretariat, Agriculture and Agri food, Canada. (http://www.agr.gc.ca/eng/industry-markets-and-trade/statistics-and-market-information/agriculture-and-food-market-information-by-region/united-states-and-mexico/market-intelligence/organic-beverage-opportunities-in-the-united-states/?id=1410083148538); accessed May 2016.

Gowda, G.N., Ijare, O.B., Shanaiah, N., Bezabeh, T., 2009. Combining nuclear magnetic resonance spectroscopy and mass spectrometry in biomarker discovery. Biomarkers in Medicine 3(3), 307-322.

Gregory, P., Hein, D., Malesker, M.A., Morrow, L.E., 2015. Over the Counter Nutritional Supplements: Implications for Critically Ill Patients. Diet and Nutrition in Critical Care, 1005-1016.

Hall, R.D., 2011. Plant Metabolomics in a Nutshell: Potential and Future Challenges. Annual Review of Plant Biology, 1-24.

Han, E.Y., Lee, B.M., Bae, J.Y., Ahn, I.Y., Lim, S.K., Kwon, M.J., Kim, S.M. and Cho, M.C., 2011. Proteo-metabolomics for nephrotoxicity biomarkers research. Toxicology Letters 205:S217

Hanigan, M.H., Deng, M., Zhang, L., Taylor, P.T., Jr., Lapus, M.G., 2005. Stress response inhibits the nephrotoxicity of cisplatin. American journal of physiology. Renal Physiology 288(1), F125-132.

Hara, M., Yoshida, M., Nishijima, H., Yokosuka, M., Iigo, M., Ohtani-Kaneko, R., Shimada, A., Hasegawa, T., Akama, Y., Hirata, K., 2001. Melatonin, a pineal secretory product with antioxidant properties, protects against cisplatin-induced nephrotoxicity in rats. Journal of Pineal Research 30(3), 129-138.

Harrigan, G.G., Goodacre, R., 2012. Metabolic profiling: its role in biomarker discovery and gene function analysis. Springer Science & Business Media.

Heyman, H.M., Senejoux, F., Seibert, I., Klimkait, T., Maharaj, V.J., Meyer, J.J.M., 2015. Identification of anti-HIV active dicaffeoylquinic- and tricaffeoylquinic acids in Helichrysum populifolium by NMR-based metabolomic guided fractionation. Fitoterapia 103, 155-164.

Hillwig, M.L., Hammer, K.D., Birt, D.F., Wurtele, E.S., 2008. Characterizing the metabolic fingerprint and anti-inflammatory activity of Hypericum gentianoides. Journal of Agricultural and Food Chemistry 56(12), 4359-4366.

Ho, C.-H., Noryati, I., Sulaiman, S.-F., Rosma, A., 2010. In vitro antibacterial and antioxidant activities of Orthosiphon stamineus Benth. extracts against food-borne bacteria. Food Chemistry 122(4), 1168-1172.

Hossain, M., Barry-Ryan, C., Martin-Diana, A.B., Brunton, N., 2010. Effect of drying method on the antioxidant capacity of six Lamiaceae herbs. Food Chemistry 123(1), 85-91.

Hossain, M.A., Ismail, Z., 2013. Isolation and characterization of triterpenes from the leaves of Orthosiphon stamineus. Arabian Journal of Chemistry 6(3), 295-298.

Hossain, M.A., Ismail, Z., Rahman, A., Kang, S.C., 2008. Chemical composition and anti-fungal properties of the essential oils and crude extracts of Orthosiphon stamineus Benth. Industrial Crops and Products 27(3), 328-334.

Humanes, B., Lazaro, A., Camano, S., Moreno-Gordaliza, E., Lazaro, J.A., Blanco-Codesido, M., Lara, J.M., Ortiz, A., Gomez-Gomez, M.M., Martin-Vasallo, P., Tejedor, A., 2012. Cilastatin protects against cisplatin-induced nephrotoxicity without compromising its anticancer efficiency in rats. Kidney International 82(6), 652-663.

Igarashi, K., Ueda, S., Yoshida, K., Kashiwagi, K., 2006. Polyamines in renal failure. Amino Acids 31(4):477-483

Ji, H., Du, A., Zhang, L., Xu, C., Yang, M., Li, F., 2012. Effects of drying methods on antioxidant properties in Robinia pseudoacacia L. flowers. Journal of Medicinal Plants Research 6(16), 3233-3239.

Kannappan, N., Madhukar, A., Mariymmal, P., Uma, S.R., Mannavalan, R., International Journal of PharmTech Research 2010 (2), 209-215

Karimi, G., Ramezani, M., Tahoonian, Z., 2005. Cisplatin nephrotoxicity and protection by milk thistle extract in rats. Evidence-Based Complementary and Alternative Medicine: eCAM 2(3), 383-386.

Karthivashan, G., Tangestani Fard, M., Arulselvan, P., Abas, F., Fakurazi, S., 2013. Identification of Bioactive Candidate Compounds Responsible for Oxidative Challenge from Hydro-Ethanolic Extract of Moringa oleifera Leaves. Journal of Food Science 78(9), C1368-C1375.

Kawai, Y., Nakao, T., Kunimura, N., Kohda, Y., Gemba, M., 2006. Relationship of intracellular calcium and oxygen radicals to cisplatin-related renal cell injury. Journal of Pharmacological Sciences 100, 65-72.

Kemsley, E. K., 1996. Discriminant analysis of high-dimensional data: a comparison of principal components analysis and partial least squares data reduction methods. Chemometrics and Intelligent Laboratory Systems 33, 47–61.

Kennedy, G.L., Ferenz, R.L., Burgess, B.A., 1986. Estimation of acute oral toxicity in rates by determination of the approximate lethal dose rather than the LD50. Journal of Applied Toxicology 6(3), 145-148.

Keun, H.C., 2006. Metabonomic modeling of drug toxicity. Pharmacology and Therapeutics 109(1-2), 92-106.

Khoo, L.W., Mediani, A., Zolkeflee, N.K.Z., Leong, S.W., Ismail, I.S., Khatib, A., Shaari, K., Abas, F., 2015. Phytochemical diversity of Clinacanthus nutans extracts and their bioactivity correlations elucidated by NMR based metabolomics. Phytochemistry Letters 14, 123-133.

Kim, K.B., Um, S.Y., Chung, M.W., Jung, S.C., Oh, J.S., Kim, S.H., Na, H.S., Lee, B.M., Choi, K.H., 2010. Toxicometabolomics approach to urinary biomarkers for mercuric chloride HgCl2-induced nephrotoxicity using proton nuclear magnetic resonance 1H NMR in rats. Toxicology and Applied Pharmacology 249(2):114-26

Kim, H.K., Choi, Y.H., Verpoorte, R., 2010. NMR-based metabolomic analysis of plants. Nature Protocols 5(3), 536-549.

Kim, H.K., Choi, Y.H., Verpoorte, R., 2011. NMR-based plant metabolomics: where do we stand, where do we go?. Trends in Biotechnology 29(6), 267-275.

Kim, H.K., Khan, S., Wilson, E.G., Kricun, S.D.P., Meissner, A., Goraler, S., Deelder, A.M., Choi, Y.H., Verpoorte, R., 2010. Metabolic classification of South American Ilex species by NMR-based metabolomics. Phytochemistry 71(7), 773-784.

Kim, Y.-H., Kim, Y.-W., Oh, Y.-J., Back, N.-I., Chung, S.-A., Chung, H.-G., Jeong, T.-S., Choi, M.-S., Lee, K.-T., 2006. Protective effect of the ethanol extract of the roots of Brassica rapa on cisplatin-induced nephrotoxicity in LLC-PK1 cells and rats. Biological and Pharmaceutical Bulletin 29(12), 2436-2441.

Kimura, T., Takabatake, Y., Takahashi, A., Kaimori, J.Y., Matsui, I., Namba, T., Kitamura, H., Niimura, F., Matsusaka, T., Soga, T., Rakugi, H., 2011. Autophagy protects the proximal tubule from degeneration and acute ischemic injury. Journal of the American Society of Nephrology 22(5):902-913

Krishnan, P., Kruger, N.J., Ratcliffe, R.G., 2005. Metabolite fingerprinting and profiling in plants using NMR. Journal of Experimental Botany 56 (410), 255-265.

Ku, K.M., Choi, J.N., Kim, J., Kim, J.K., Yoo, L.G., Lee, S.J., Hong, Y.S., Lee, C.H., 2010. Metabolomics analysis reveals the compositional differences of shade grown tea (Camellia sinensis L.). Journal of Agricultural and Food Chemistry 58(1), 418-426.

Lee, B.M., Lim, S.K., Han, E.Y., Bae, J.Y., Ahn, I.Y., Kwon, M.J., Kim, S.M., Cho, M.C., 2011. Toxicogeno-metabolomics approach for the discovery of nephrotoxicity biomarkers. Toxicology Letters 205:S215

Lee, E.J., Kim, J.S., Kim, H.P., Lee, J.-H., Kang, S.S., 2010. Phenolic constituents from the flower buds of Lonicera japonica and their 5-lipoxygenase inhibitory activities. Food Chemistry 120(1), 134-139.

Lee, J.E., Lee, B.J., Chung, J.O., Kim, H.N., Kim, E.H., Jung, S., Lee, H., Lee, S.J., Hong, Y.S., 2015. Metabolomic unveiling of a diverse range of green tea (Camellia sinensis) metabolites dependent on geography. Food Chemistry 174, 452-459.

Lenz, E.M., Wilson, I.D., 2007. Analytical strategies in metabonomics. Journal of Proteome Research 6(2), 443-458.

Li, Z.Y., Zhi, H.J., Xue, S.Y., Sun, H.F., Zhang, F.S., Jia, J.P., Xing, J., Zhang, L.Z., Qin, X.M., 2012. Metabolomic profiling of the flower bud and rachis of Tussilago farfara with antitussive and expectorant effects on mice. Journal of Ethnopharmacology 140(1), 83-90.

Li, Z.Y., Zhi, H.J., Zhang, F.S., Sun, H.F., Zhang, L.Z., Jia, J.P., Xing, J., Qin, X.M., 2013. Metabolomic profiling of the antitussive and expectorant plant Tussilago farfara L. by nuclear magnetic resonance spectroscopy and multivariate data analysis. Journal of Pharmaceutical and Biomedical Analysis 75, 158-164.

Liang, Y.-S., Choi, Y.H., Kim, H.K., Linthorst, H.J., Verpoorte, R., 2006. Metabolomic analysis of methyl jasmonate treated Brassica rapa leaves by 2-dimensional NMR spectroscopy. Phytochemistry 67(22), 2503-2511.

Lindon, J.C., Nicholson, J.K., Holmes, E., 2011. The handbook of metabonomics and metabolomics. Elsevier

Lippert, B., 1999. Cisplatin: chemistry and biochemistry of a leading anticancer drug. John Wiley & Sons.

Ludwig, C., Viant, M.R., 2010. Two-dimensional J-resolved NMR spectroscopy: review of a key methodology in the metabolomics toolbox. Phytochemical Analysis 21(1), 22-32.

Maheswary, C., Mariyammal, Venkatnarayanan, R., International Journal of Pharmacy and Technology 2011 (3), 1584-1592.

Mahrous, E.A., Farag, M.A., 2015. Two dimensional NMR spectroscopic approaches for exploring plant metabolome: A review. Journal of Advanced Research 6(1), 3-15.

Martin, F.P.J., Wang, Y., Sprenger, N., Yap, I.K., Lundstedt, T., Lek, P., Rezzi, S., Ramadan, Z., van Bladeren, P., Fay, L.B., Kochhar, S., 2008. Probiotic modulation of symbiotic gut microbial–host metabolic interactions in a humanized microbiome mouse model. Molecular Systems Biology 4(1)

Masuda, T., Masuda, K., Shiragami, S., Jitoe, A., Nakatani, N., 1992. Orthosiphol A and B, novel diterpenoid inhibitors of TPA (12-O-tetradecanoylphorbol-13-acetate)-induced inflammation, from Orthosiphon stamineus. Tetrahedron 48(33), 6787-6792.

Matsubara, T., Bohgaki, T., Watarai, M., Suzuki, H., Ohashi, K., Shibuya, H., 1999. Antihypertensive actions of methylripariochromene A from Orthosiphon aristatus, an Indonesian traditional medicinal plant. Biological and Pharmaceutical Bulletin 22(10), 1083-1088.

Mediani, A., Abas, F., Khatib, A., Tan, C.P., Ismail, I.S., Shaari, K., Ismail, A., Lajis, N., 2015. Phytochemical and biological features of Phyllanthus niruri and Phyllanthus urinaria harvested at different growth stages revealed by 1H NMR-based metabolomics. Industrial Crops and Products 77, 602-613.

Mihaleva, V.V., te Beek, T.A., van Zimmeren, F., Moco, S., Laatikainen, R., Niemitz, M., Korhonen, S.-P., van Driel, M.A., Vervoort, J., 2013. MetIDB: a publicly accessible database of predicted and experimental 1H NMR spectra of flavonoids. Analytical Chemistry 85(18), 8700-8707.

Miller, R.P., Tadagavadi, R.K., Ramesh, G., Reeves, W.B., 2010. Mechanisms of Cisplatin nephrotoxicity. Toxins 2(11), 2490-2518.

Mohamed, E.A., Lim, C.P., Ebrika, O.S., Asmawi, M.Z., Sadikun, A., Yam, M.F., 2011a. Toxicity evaluation of a standardised 50% ethanol extract of Orthosiphon stamineus. Journal of Ethnopharmacology 133(2), 358-363.

Mohamed, E.A., Mohamed, A.J., Asmawi, M.Z., Sadikun, A., Ebrika, O.S., Yam, M.F., 2011b. Antihyperglycemic effect of Orthosiphon stamineus Benth leaves extract and its bioassay-guided fractions. Molecules 16(5), 3787-3801.

Mohamed, E.A., Yam, M.F., Ang, L.F., Mohamed, A.J., Asmawi, M.Z., 2013. Antidiabetic properties and mechanism of action of Orthosiphon stamineus Benth bioactive sub-fraction in streptozotocin-induced diabetic rats. Journal of Acupuncture and Meridian Studies 6(1), 31-40.

Mohamed, H.E., El-Swefy, S.E., Mohamed, R.H., Ghanim, A.M., 2013. Effect of erythropoietin therapy on the progression of cisplatin induced renal injury in rats. Experimental and Toxicologic Pathology 65(1-2), 197-203.

Mohan, I.K., Khan, M., Shobha, J.C., Naidu, M.U.R., Prayag, A., Kuppusamy, P., Kutala, V.K., 2006. Protection against cisplatin-induced nephrotoxicity by Spirulina in rats. Cancer Chemotherapy and Pharmacology 58(6), 802-808.

Monograph, M.H., 2009. Malaysian Herbal Monograph Committee, Kuala Lumpur, Malaysia. Vol. 2.

Mora, L., 2003. The effects of oral glutamine on cisplatin-induced nephrotoxicity in rats. Pharmacological Research 47(6), 517-522.

Muhammad, H., Gomes-Carneiro, M.R., Poca, K.S., De-Oliveira, A.C., Afzan, A., Sulaiman, S.A., Ismail, Z., Paumgartten, F.J., 2011. Evaluation of the genotoxicity of Orthosiphon stamineus aqueous extract. Journal of Ethnopharmacology 133(2), 647-653.

Mujumdar, A.S. and Law, C.L., 2010. Drying technology: Trends and applications in postharvest processing. Food and Bioprocess Technology 3(6), 843-852.

Naughton, C.A., 2008. Drug-induced nephrotoxicity. American Family Physician 78(6), 743-750

Nazıroğlu, M., Karaoğlu, A., Aksoy, A.O., 2004. Selenium and high dose vitamin E administration protects cisplatin-induced oxidative damage to renal, liver and lens tissues in rats. Toxicology 195(2), 221-230.

Neergheen-Bhujun, V.S., 2013. Underestimating the toxicological challenges associated with the use of herbal medicinal products in developing countries. BioMed Research International, 9 (Article ID 804086) .

Newman, D.J., Cragg, G.M., 2012. Natural products as sources of new drugs over the 30 years from 1981 to 2010. Journal of Natural Products 75(3), 311-335.

Newman, D.J., Cragg, G.M., 2016. Natural products as sources of new drugs from 1981 to 2014. Journal of Natural Products 79(3), 629-661.

Nguyen, M.T.T., Suresh Awale, Tezuka, Y., Chien-Hsiung, C., Kadota, S., 2004. Staminane- and Isopimarane-Type Diterpenes from Orthosiphon stamineus of Taiwan and Their Nitric Oxide Inhibitory Activity. Journal of Natural Products 67, 654-658.

Nicholson, J.K., Lindon, J.C., 2008. Systems biology: metabonomics. Nature 455(7216), 1054-1056.

Niemann, C.U. and Serkova, N.J., 2007. Biochemical mechanisms of nephrotoxicity-application for metabolomics. Expert Opinion on Drug Metabolism and Toxicology 3(4):527-544

Niu, Q.Y., Li, Z.Y., Du, G.H., Qin, X.M., 2016. 1H NMR based metabolomic profiling revealed doxorubicin-induced systematic alterations in a rat model. Journal of Pharmaceutical and Biomedical Analysis 118:338-348

NovoaCarballal, R., FernandezMegi a, E., Jimenez, C., Riguera, R., 2011. NMR methods for unravelling the spectra of complex mixtures. Natural product reports 28(1), 78-98.

Nuengchamnong, N., Krittasilp, K., Ingkaninan, K., 2011. Characterisation of phenolic antioxidants in aqueous extract of Orthosiphon grandiflorus tea by LC-ESI-MS/MS coupled to DPPH assay. Food Chemistry 127(3), 1287-1293.

OECD, 1992. OECD Guideline For Testing Of Chemicals. Organization for Economic Cooperation and Development, Paris, France. 420.

Ohashi, K., Bohgaki, T., Shibuya, H., 2000. Antihypertensive substance in the leaves of kumis kucing (Orthosiphon aristatus) in Java Island. Yakugaku zasshi: Journal of the Pharmaceutical Society of Japan 120(5), 474-482.

Olah, N.-K., Radu, L., Mogoşan, C., Hanganu, D., Gocan, S., 2003. Phytochemical and pharmacological studies on Orthosiphon stamineus Benth. (Lamiaceae) hydroalcoholic extracts. Journal of Pharmaceutical and Biomedical Analysis 33(1), 117-123.

Pabla, N., Dong, Z., 2008. Cisplatin nephrotoxicity: mechanisms and renoprotective strategies. Kidney International 73(9), 994-1007.

Pace, A., Savarese, A., Picardo, M., Maresca, V., Pacetti, U., Del Monte, G., Biroccio, A., Leonetti, C., Jandolo, B., Cognetti, F., 2003. Neuroprotective effect of vitamin E supplementation in patients treated with cisplatin chemotherapy. Journal of Clinical Oncology 21(5), 927-931.

Park, M.H., Igarashi, K., 2013. Polyamines and their metabolites as diagnostic markers of human diseases. Biomolecules and Therapeutics 21(1):1-9

Patti, G.J., Yanes, O., Siuzdak, G., 2012. Innovation: Metabolomics: the apogee of the omics trilogy. Nature Reviews Molecular Cell Biology 13(4), 263-269.

Paudel, L., Wyzgoski, F.J., Giusti, M.M., Johnson, J.L., Rinaldi, P.L., Scheerens, J.C., Chanon, A.M., Bomser, J.A., Miller, A.R., Hardy, J.K., Reese, R.N., 2014. NMR-Based Metabolomic Investigation of Bioactivity of Chemical Constituents in Black Raspberry (Rubus occidentalis L.) Fruit Extracts. Journal of Agricultural and Food Chemistry 62(8), 1989-1998.

Petersen., M., and Simmonds, M.S., 2003. Rosmarinic acid. Phytochemistry 62(2), 121-125.

Pietta, P., Mauri, P., Gardana, C., Bruno, A., 1991. High-performance liquid chromatography with diode-array ultraviolet detection of methoxylated flavones in Orthosiphon leaves. Journal of Chromatography A 547, 439-442.

Portilla, D., Li, S., Nagothu, K.K., Megyesi, J., Kaissling, B., Schnackenberg, L., Safirstein, R.L., Beger, R.D., 2006. Metabolomic study of cisplatin-induced nephrotoxicity. Kidney International 69(12), 2194-2204.

Ramirez, T., Daneshian, M., Kamp, H., Bois, F.Y., Clench, M.R., Coen, M., Donley, B., Fischer, S.M., Ekman, D.R., Fabian, E., 2013. Metabolomics in toxicology and preclinical research. Altex 30(2), 209.

Rasmussen, L., Savorani, F., Larsen, T., Dragsted, L., Astrup, A., Engelsen, S. 2011. Standardization of factors that influence human urine metabolomics. Metabolomics 7, 71–83.

Robertson, D.G., Watkins, P.B., Reily, M.D., 2011. Metabolomics in toxicology: preclinical and clinical applications. Toxicological sciences 120 Suppl 1, S146-170.

Robinette, S.L., Brüschweiler, R., Schroeder, F.C. and Edison, A.S., 2011. NMR in metabolomics and natural products research: two sides of the same coin. Accounts of Chemical Research 45(2), 288-297.

Sahib, H., Ismail, Z., Othman, N., Majid, A.A., 2009. Orthosiphon stamineus Benth. methanolic extract enhances the anti-proliferative effects of tamoxifen on human hormone dependent breast cancer. International Journal of Pharmacology 5(4), 273-276.

Santos, N.A., Catao, C.S., Martins, N.M., Curti, C., Bianchi, M.L., Santos, A.C., 2007. Cisplatin-induced nephrotoxicity is associated with oxidative stress, redox state unbalance, impairment of energetic metabolism and apoptosis in rat kidney mitochondria. Archives of Toxicology 81(7), 495-504.

Santoso, J.T., Lucci, J.A., 3rd, Coleman, R.L., Schafer, I., Hannigan, E.V., 2003. Saline, mannitol, and furosemide hydration in acute cisplatin nephrotoxicity: a randomized trial. Cancer Chemotherapy and Pharmacology 52(1), 13-18.

Sarimeseli, A., 2011. Microwave drying characteristics of coriander (Coriandrum sativum L.) leaves. Energy Conversion and Management 52(2), 1449-1453.

Schripsema, J., 2010. Application of NMR in plant metabolomics: techniques, problems and prospects. Phytochemical Analysis 21(1), 14-21.

Schut, G., Zwaving, J., 1993. Pharmacological investigation of some lipophilic flavonoids from Orthosiphon aristatus. Fitoterapia 64, 99-102.

Sellami, I.H., Wannes, W.A., Bettaieb, I., Berrima, S., Chahed, T., Marzouk, B., Limam, F., 2011. Qualitative and quantitative changes in the essential oil of Laurus nobilis L. leaves as affected by different drying methods. Food Chemistry 126(2), 691-697.

Sellers, R.S., Mortan, D., Michael, B., Roome, N., Johnson, J.K., Yano, B.L., Perry, R., Schafer, K., 2007. Society of Toxicologic Pathology position paper: organ weight recommendations for toxicology studies. Toxicologic Pathology 35(5), 751-755.

Şener, G., Şatiroglu, H., Kabasakal, L., Arbak, S., Öner, S., Ercan, F., Keyer-Uysal, M., 2000. The protective effect of melatonin on cisplatin nephrotoxicity. Fundamental and Clinical Pharmacology 14(6), 553-560.

Shyur, L.F., Yang, N.S., 2008. Metabolomics for phytomedicine research and drug development. Current Opinion in Chemical Biology 12(1), 66-71.

Sogi, D.S., Siddiq, M., Greiby, I., Dolan, K.D., 2013. Total phenolics, antioxidant activity, and functional properties of 'Tommy Atkins' mango peel and kernel as affected by drying methods. Food Chemistry 141(3), 2649-2655.

Son, J.Y., 2011. Orthosiphon stamineus Reduces Appetite and Visceral Fat in Rats. Journal of the Korean Society for Applied Biological Chemistry 54(2).

Stampoulis, P., Tezuka, Y., Banskota, A.H., Tran, K.Q., Saiki, I., Kadota, S., 1999a. Staminol A, a Novel Diterpene from Orthosiphon stamineus. Tetrahedron Letters 40, 4239-4242.

Stampoulis, P., Tezuka, Y., Banskota, A.H., Tran, K.Q., Saiki, I., Kadota, S., 1999b. Staminolactones A and B and Norstaminol A: Three Highly Oxygenated Staminane-Type Diterpenes from Orthosiphon stamineus. Organic Letters 1(9), 1367-1370.

Stankovic, M.S., Niciforovic, N., Topuzovic, M., Solujic, S., 2011. Total phenolic content, flavonoid concentrations and antioxidant activity, of the whole plant and plant parts extracts from Teucrium montanum L. var. montanum, f. supinum (L.) Reichenb. Biotechnology and Biotechnological Equipment 25(1), 2222-2227.

Sumaryono, W., Proksch, P., Wray, V., Witte, L., Hartmann, T., 1991. Qualitative and quantitative analysis of the phenolic constituents from Orthosiphon aristatus. Planta Medica 57(02), 176-180.

Suzuki, R., Hasuike, Y., Hirabayashi, M., Fukuda, T., Okada, Y., Shirataki, Y., 2013. Identification of a xanthine oxidase-inhibitory component from Sophora flavescens using NMR-based metabolomics. Natural Product Communications 8(10), 1409-1412.

Takeda, Y., Matsumoto, T., Terao, H., Shingu, T., Futatsuishi, Y., Nohara, T., Kajmoto, T., 1993. Orthosiphol D and E, minor diterpenes from Orthosiphon stamineus. Phytochemistry 33(2), 411-415.

Tapp, H. S., and Kemsley, E. K., 2009. Notes on the practical utility of OPLS. Trends in Analytical Chemistry 28, 1322–1327.

Terpinc, P., Čeh, B., Ulrih, N.P., Abramovič, H., 2012. Studies of the correlation between antioxidant properties and the total phenolic content of different oil cake extracts. Industrial Crops and Products 39, 210-217.

Tezuka, P, S., AH, B., S, A., KQ, T., I, S., S., K., 2000. Constituents of the vietnamese medicinal plant Orthosiphon stamineus. Chemical and Pharmaceutical Bulletin 48(11), 1711-1719.

Thukral, S.K., Nordone, P.J., Hu, R., Sullivan, L., Galambos, E., Fitzpatrick, V.D., Healy, L., Bass, M.B., Cosenza, M.E., Afshari, C.A., 2005. Prediction of nephrotoxicant action and identification of candidate toxicity-related biomarkers. Toxicologic Pathology 33(3):343-355

Torras-Claveria, L., Berkov, S., Jáuregui, O., Caujapé, J., Viladomat, F., Codina, C., Bastida, J., 2010. Metabolic profiling of bioactive Pancratium canariense extracts by GC-MS. Phytochemical Analysis 21(1), 80-88.

Trygg, J., and Wold, S., 2002. Orthogonal projections to latent structures (O-PLS). Journal of Chemometrics 16, 119–128.

Tsuruya, K., Tokumoto, M., Ninomiya, T., Hirakawa, M., Masutani, K., Taniguchi, M., Fukuda, K., Kanai, H., Hirakata, H., Iida, M., 2003. Antioxidant ameliorates cisplatin-induced renal tubular cell death through inhibition of death receptor-mediated pathways. American Journal of Physiology-Renal Physiology 285(2), F208-F218.

Uehara, T., Horinouchi, A., Morikawa, Y., Tonomura, Y., Minami, K., Ono, A., Yamate, J., Yamada, H., Ohno, Y., Urushidani, T., 2014 Identification of metabolomic biomarkers for drug-induced acute kidney injury in rats. Journal of applied Toxicology : JAT 34(10):1087-95

Ueki, M., Ueno, M., Morishita, J., Maekawa, N., 2013. Curcumin ameliorates cisplatin-induced nephrotoxicity by inhibiting renal inflammation in mice. Journal of Bioscience and Bioengineering 115(5), 547-551.

Ulrich-Merzenich, G., Panek, D., Zeitler, H., Wagner, H., Vetter, H., 2009. New perspectives for synergy research with the “omic”-technologies. Phytomedicine 16(6), 495-508.

Van De Poll, M.C., Soeters, P.B., Deutz, N.E., Fearon, K.C., Dejong, C.H., 2004. Renal metabolism of amino acids: its role in interorgan amino acid exchange. The American Journal of Clinical Nutrition 79(2):185-197

Van der Kooy, F., Maltese, F., Choi, Y.H., Kim, H.K., Verpoorte, R., 2009. Quality control of herbal material and phytopharmaceuticals with MS and NMR based metabolic fingerprinting. Planta Medica 75(7), 763-775.

Verpoorte, R., Choi, Y.H., Kim, H.K., 2007. NMR-based metabolomics at work in phytochemistry. Phytochemistry Reviews 6(1), 3-14.

Vogelgesang, B., Abdul-Malak, N., Reymermier, C., Altobelli, C., Saget, J., 2011. On the effects of a plant extract of Orthosiphon stamineus on sebum-related skin imperfections. International Journal of Cosmetic Science 33(1), 44-52.

Wang, J., van der Heijden, R., Spruit, S., Hankermeier, T., Chan, K., van der Greef, J., Xu, G., Wang, M., 2009. Quality and safety of Chinese herbal medicines guided by a systems biology perspective. Journal of Ethnopharmacology 126(1), 31-41.

Wang, M., Lamers, R.J.A., Korthout, H.A., van Nesselrooij, J.H., Witkamp, R.F., van der Heijden, R., Voshol, P.J., Havekes, L.M., Verpoorte, R., van der Greef, J., 2005. Metabolomics in the context of systems biology: bridging traditional Chinese medicine and molecular pharmacology. Phytotherapy Research 19, 173-182.

Waters, N.J., Waterfield, C.J., Farrant, R.D., Holmes, E., Nicholson, J.K., 2005. Metabonomic deconvolution of embedded toxicity: application to thioacetamide hepato-and nephrotoxicity. Chemical Research in Toxicology 18, 639-654.

Weijl, N., Elsendoorn, T., Lentjes, E., Hopman, G., Wipkink-Bakker, A., Zwinderman, A., Cleton, F., Osanto, S., 2004. Supplementation with antioxidant micronutrients and chemotherapy-induced toxicity in cancer patients treated with cisplatin-based chemotherapy: a randomised, double-blind, placebo-controlled study. European Journal of Cancer 40(11), 1713-1723.

Weiss, R.H., Kim, K., 2012. Metabolomics in the study of kidney diseases. Nature Reviews Nephrology 8(1), 22-33.

Wen, H., Yang, H.J., Choi, M.J., Kwon, H.N., Kim, M.A., Hong, S.S., Park, S.H., 2011. Identification of Urinary Biomarkers Related to Cisplatin-Induced Acute Renal Toxicity Using NMR-Based Metabolomics. Biomolecules and Therapeutics 19(1):38-44.

Westerhuis, J., Hoefsloot, H. J., Smit, S., Vis, D., Smilde, A., Van Velzen, E. J., van Duijnhoven, J.P. and van Dorsten, F.A., 2008. Assessment of PLS-DA cross validation. Metabolomics 4, 81–89

Wishart, D.S., 2008. Quantitative metabolomics using NMR. Trends in Analytical Chemistry 27(3), 228-237.

Wojdyło, A., Figiel, A., Lech, K., Nowicka, P., Oszmiański, J., 2014. Effect of Convective and Vacuum–Microwave Drying on the Bioactive Compounds, Color, and Antioxidant Capacity of Sour Cherries. Food and Bioprocess Technology 7(3), 829-841.

Wolfender, J.-L., Rudaz, S., Hae Choi, Y., Kyong Kim, H., 2013. Plant metabolomics: from holistic data to relevant biomarkers. Current Medicinal Chemistry 20(8), 1056-1090.

Worley, B., and Powers, R., 2013. Multivariate analysis in metabolomics. Current Metabolomics 1(1):92

Wu, Y.J., Muldoon, L.L., Neuwelt, E.A., 2005. The chemoprotective agent N-acetylcysteine blocks cisplatin-induced apoptosis through caspase signaling pathway. Journal of Pharmacology and Experimental Therapeutics 312(2), 424-431.

Xi, Y., de Ropp, J.S., Viant, M.R., Woodruff, D.L., Yu, P., 2008. Improved identification of metabolites in complex mixtures using HSQC NMR spectroscopy. Analytica Chimica Acta 614(2), 127-133.

Xu, E.Y., Perlina, A., Vu, H., Troth, S.P., Brennan, R.J., Aslamkhan, A.G., Xu, Q., 2008. Integrated pathway analysis of rat urine metabolic profiles and kidney transcriptomic profiles to elucidate the systems toxicology of model nephrotoxicants. Chemical Research in Toxicology 21(8):1548-1561

Yam, M.F., Ang, L.F., Basir, R., Salman, I.M., Ameer, O.Z., Asmawi, M.Z., 2009. Evaluation of the anti-pyretic potential of Orthosiphon stamineus Benth standardized extract. Inflammopharmacology 17(1), 50-54.

Yam, M.F., Ang, L.F., Salman, I.M., Ameer, O.Z., Lim, V., Ong, L.M., Ahmad, M., Asmawi, M.Z., Basir, R., 2009. Orthosiphon stamineus leaf extract protects against ethanol-induced gastropathy in rats. Journal of Medicinal Food 12(5), 1089-1097.

Yam, M.F., Asmawi, M.Z., Basir, R., 2008. An investigation of the anti-inflammatory and analgesic effects of Orthosiphon stamineus leaf extract. Journal of Medicinal Food 11(2), 362-368.

Yam, M.F., Basir, R., Asmawi, M.Z., Ismail, Z., 2007. Antioxidant and hepatoprotective effects of Orthosiphon stamineus Benth. standardized extract. The American Journal of Chinese Medicine 35(1), 12.

Yam, M.F., Lim, C.P., Fung Ang, L., Por, L.Y., Wong, S.T., Asmawi, M.Z., Basir, R., Ahmad, M., 2013. Antioxidant and toxicity studies of 50% methanolic extract of Orthosiphon stamineus Benth. BioMed Research International 2013, 351602.

Yam, M.F., Mohamed, E.A., Ang, L.F., Pei, L., Darwis, Y., Mahmud, R., Asmawi, M.Z., Basir, R., Ahmad, M., 2012. A simple isocratic HPLC method for the simultaneous determination of sinensetin, eupatorin, and 3'-hydroxy-5,6,7,4'-tetramethoxyflavone in Orthosiphon stamineus extracts. Journal of Acupuncture and Meridian Studies 5(4), 176-182.

Yang, S.Y., Kim, H.K., Lefeber, A.W., Erkelens, C., Angelova, N., Choi, Y.H., Verpoorte, R., 2006. Application of two-dimensional nuclear magnetic resonance spectroscopy to quality control of ginseng commercial products. Planta Medica 72(4), 364-369.

Yao, X., Panichpisal, K., Kurtzman, N., Nugent, K., 2007. Cisplatin Nephrotoxicity: A Review. The American Journal of the Medical Sciences 334(2), 10.

Yin, P., Peter, A., Franken, H., Zhao, X., Neukamm, S. S., Rosenbaum, L., Lucio, M., Zell, A., Häring, H.U., Xu, G., Lehmann, R., 2013. Preanalytical aspects and sample quality assessment in metabolomics studies of human blood. Clinical Chemistry. 59, 833–845.

Yuliana, N.D., Jahangir, M., Verpoorte, R., Choi, Y.H., 2013. Metabolomics for the rapid dereplication of bioactive compounds from natural sources. Phytochemistry Reviews 12(2), 293-304.

Yuliana, N.D., Khatib, A., Link-Struensee, A.M., Ijzerman, A.P., Rungkat-Zakaria, F., Choi, Y.H., Verpoorte, R., 2009. Adenosine A1 receptor binding activity of methoxy flavonoids from Orthosiphon stamineus. Planta Medica 75(2), 132-136.

Yuliana, N.D., Khatib, A., Verpoorte, R., Choi, Y.H., 2011. Comprehensive extraction method integrated with NMR metabolomics: a new bioactivity screening method for plants, adenosine A1 receptor binding compounds in Orthosiphon stamineus Benth. Analytical chemistry 83(17), 6902-6906.

Zeng, M., Liang, Y., Li, H., Wang, M., Wang, B., Chen, X., Zhou, N., Cao, D., Wu, J., 2010. Plasma metabolic fingerprinting of childhood obesity by GC/MS in conjunction with multivariate statistical analysis. Journal of Pharmaceutical and Biomedical Analysis 52(2), 265-272.

Zhang, A.H., Sun, H. and Wang, X.J., 2014. Potential Applications and Development of Cell Metabolomics in Natural Products. Journal of Drug Metabolism and Toxicology 5,163.

Zhang, A., Sun, H., Wang, P., Han, Y., Wang, X., 2012. Modern analytical techniques in metabolomics analysis. Analyst 137(2), 293-300.

Zhong, Y.S., Yu, C.H., Ying, H.Z., Wang, Z.Y., Cai, H.F., 2012. Prophylactic effects of Orthosiphon stamineus Benth. extracts on experimental induction of calcium oxalate nephrolithiasis in rats. Journal of Ethnopharmacology 144(3), 761-767.

BIODATA OF STUDENT

Raghunath Pariyani was born on 15th May, 1983 in Palakkad, Kerala, India. He did his early schooling in Palakkad. In the year 2000, after passing pre-degree examination, he joined University of Calicut for Bachelor degree in Pharmacy (B. Pharm). In 2005, the degree was awarded with gold medal for securing the highest marks in University of Calicut B. Pharm examinations, and then he registered as a Pharmacist in Kerala State Pharmacy Council. After securing a state merit scholarship from the Government of Kerala, he joined in University of Kerala in 2006 to further Master in Pharmacy (M. Pharm), specialized in Pharmaceutical Chemistry, and completed in 2008. During M. Pharm, he gained experience as a toxicological analyst and pursued the research on evaluation of antimycobacterial activity of selected compounds. After which, he started career as Lecturer in Pharmacy in Masterskill University College of Nursing & Health (renamed as Asia Metropolitan University), Malaysia, until 2011. His passion towards the research in natural product sources as potential drug and health supplements led to join for PhD, through which he aimed to develop and enhance the systematic research aptitude. He started the PhD research on September 2011 at Laboratory of Natural Products, Institute of Bioscience, Universiti Putra Malaysia, in *Orthosiphon stamineus,* which is a traditional herb as well as health supplement. He applied NMR-based metabolomics approach in studying the chemical and biological properties of *Orthosiphon stamineus,* the results of which are presented in this thesis.

LIST OF PUBLICATIONS

Pariyani, R., Safinar Ismail, I., Azam, A.A., Abas, F., Shaari, K., Sulaiman, M.R., 2015. Phytochemical Screening and Acute Oral Toxicity Study of Java Tea Leaf Extracts. BioMed research international 2015.

Pariyani, R., Ismail, I.S., Azam, A., Abas, F., Shaari, K., Hamza, H., 2016. Urinary metabolic profiling of cisplatin nephrotoxicity and nephroprotective effects of Orthosiphon stamineus leaves elucidated by ^{1}H NMR spectroscopy. Journal of Pharmaceutical and Biomedical Analysis (135), 20-30.

Pariyani, R., Ismail, I.S., Azam, A.A., Abas, F. and Shaari, K., 2017. Identification of the compositional changes in *Orthosiphon stamineus* leaves triggered by different drying techniques using ^{1}H NMR metabolomics. Journal of the Science of Food and Agriculture.

Conference Presentations & Awards

P Raghunath, IS Ismail., Discriminating the effect of different drying methods on the biological and chemical properties of Java tea through metabolomics approach, The TriSys Asian Regional Conference on Systems Biology 2015 (ARCSB), Bangi, Malaysia, 08 - 09 September 2015, (*Best Young researcher presentation award)*

P Raghunath, IS Ismail, Amalina Ahmad Azam, Alfi Khatib, Faridah Abas and Khozirah Shaari, Metabolomic study on the effect of *Orthosiphon stamineus* leaf extracts in cisplatin-induced nephrotoxicity, Inaugural Symposium of the Phytochemical Society of Asia (ISPSA), Tokushima, Japan, 30 August - 2 September 2015

P Raghunath, IS Ismail., ^{1}H NMR based metabolomics approach in identifying urinary biomarkers associated with cisplatin nephrotoxicity in rat model, 3rd International Postgraduate Conference in Pharmaceutical Sciences, UiTM, Malaysia, 13-14 August 2014

UNIVERSITI PUTRA MALAYSIA

STATUS CONFIRMATION FOR THESIS / PROJECT REPORT AND COPYRIGHT

ACADEMIC SESSION: Second Semester 2016/2017

TITLE OF THESIS / PROJECT REPORT:

NUCLEAR MAGNETIC RESONANCE METABOLOMICS APPROACH IN CHEMICAL AND PROTECTIVE EVALUATIONS OF *Orthosiphon stamineus* BENTH. LEAF EXTRACTS ON CISPLATIN-INDUCED NEPHROTOXICITY

NAME OF STUDENT : RAGHUNATH PARIYANI

I acknowledge that the copyright and other intellectual property in the thesis/project report belonged to Universiti Putra Malaysia and I agree to allow this thesis/project report to be placed at the library under the following terms:

1. This thesis/project report is the property of Universiti Putra Malaysia.
2. The library of Universiti Putra Malaysia has the right to make copies for educational purposes only.
3. The library of Universiti Putra Malaysia is allowed to make copies of this thesis for academic exchange.

I declare that this thesis is classified as :

*Please tick (√)

☐	**CONFIDENTIAL**	(Contain confidential information under Official Secret Act 1972).
☐	**RESTRICTED**	(Contains restricted information as specified by the organization/institution where research was done).
☐	**OPEN ACCESS**	I agree that my thesis/project report to be published as hard copy or online open access.

This thesis is submitted for :

☐	**PATENT**	Embargo from_____ until _____ (date) (date)

Approved by:

_____	_____
(Signature of Student)	(Signature of Chairman of Supervisory Committee)
New IC No/ Passport No.:	Name:
Date :	Date :

[Note : If the thesis is CONFIDENTIAL or RESTRICTED, please attach with the letter from the organization/institution with period and reasons for confidentially or restricted.]