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METABOLOMICS ANALYSIS OF THE ANTI-INFLAMMATORY ACTIVITY OF Scurrula ferruginea (Jack) Danser PARASITIZING ON THREE DIFFERENT HOST PLANTS, AND INSIGHTS INTO POSSIBLE MECHANISM

HONG XIA

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By

HONG XIA

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

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DEDICATION

This thesis is dedicated to my beloved parents, family, and friends.

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Doctor of Philosophy

METABOLOMICS ANALYSIS OF THE ANTI-INFLAMMATORY ACTIVITY OF *Scurrula ferruginea* (Jack) Danser PARASITIZING ON THREE DIFFERENT HOST PLANTS, AND INSIGHTS INTO POSSIBLE MECHANISM

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

Scurrula ferruginea (Jack) Danser of Loranthaceae family is a hemi-parasitic shrub that grows on a dicotyledonous tree. Despite its traditional use for a long time in some disorders, there is very limited research on its bioactivities and phytochemistry, as well as, there is also no research concerning the influence of the host plants on the chemistry and bioactivity of *S. ferruginea*. The main purpose of this study was to evaluate the anti-inflammatory activity of *S. ferruginea* and the mechanism of action, as well as determine the relationship between the metabolites of *S. ferruginea* with its anti-inflammatory potency. The anti-inflammatory activity was assessed via inhibition of nitric oxide (NO) production in lipopolysaccharide (LPS) and interferon-γ (IFN-γ) induced RAW 264.7 macrophage cells. The air dried and freeze dried *S. ferruginea* parasitizing on *Vitex negundo* were investigated firstly. The results showed freeze drying was the most appropriate drying method for the anti-inflammatory activity. Subsequently, the anti-inflammatory ability was evaluated on the freeze dried leaves and stems of *S. ferruginea* parasitizing on three different hosts. The results showed that *S. ferruginea* stems parasitizing on *Tecoma stans* and *Vitex negundo* exhibited higher anti-inflammatory activity compared to the corresponding samples of harvesting from *Micromelum minutum*, and to the corresponding *S. ferruginea* leaf samples. Their IC_{50} values of the two *S. ferruginea* stems were 114.47 ± 2.96 and 118.87 ± 2.31μg/mL, respectively. Then, the anti-inflammatory mechanism was deciphered through messenger Ribonucleic acid (mRNA) and protein expression of the inflammatory cytokines, including inducible nitric oxide synthase (*i*NOS), cyclooxygenase-2 (COX-2), interleukin (IL), and tumor necrosis factor (TNF). The techniques of reverse transcriptase and real time quantitative polymerase chain reactions (RT-PCR and qPCR) were employed. The stem of *S. ferruginea* hosted on *Tecoma stans* exerts anti-inflammatory capability attributed to the suppression of *i*NOS and IL-1β mRNA expression, as well as the protein production inhibition of IL-

1β, IL-6, IL-10, and TNF-α besides NO secretion inhibition. Afterwards, the metabolite variation was examined using proton nuclear magnetic resonance (^{1}H NMR) and liquid chromatography mass spectrometry (LC-MS) combined with multivariate data analysis (MVDA). The metabolomics approach was employed in evaluating the metabolite variation and its relationship with the anti-inflammatory activity. Principal component analysis (PCA) indicated clear discriminations among the different plant parts and host plants based on the identified metabolites. Quercitrin, 4''-*O*-acetylquercitrin, catechin, gallic acid and chlorogenic acid, as well as alanine, valine, leucine, isoleucine, malic acid, succinic acid, citric acid, and acetic acid were found higher in the leaf extracts. While threonine, histidine, fumaric acid and choline were found present mainly in the stems. The stem of *S. ferruginea*-*T. stans* also possessed higher quercitrin, 4''-*O*-acetylquercitrin, catechin, valine, leucine, isoleucine, fumaric acid, gallic acid, chlorogenic acid, and sucrose than the stems of *S. ferruginea*-*V. negundo* and *S. ferruginea*-*M. minutum*. A correlation was observed from partial least square regression (PLS) model that the anti-inflammatory bioactivity might be associated with the presence of choline, isoleucine, catechin, leucine, and chlorogenic acid, as well as some unidentified metabolites such as ions mass *m/z* at 369.1157, 401.0851, 431.1327, and 473.1794. Lastly, the bioactive-activity guided fractionation by liquid-liquid extraction on freeze-dried stem of *S. ferruginea* hosted on *T. stans* was carried out. The results showed that ethyl acetate fraction displayed the highest NO inhibition with 84.8 ± 1.45% at 200 µg/mL. Chloroform fraction displayed higher activity than *n*-butanol and ethyl acetate fractions at 50 µg/mL, but $CHCl_3$ fraction showed more cytotoxicity towards the RAW 264.7 cells. The catechin was elucidated in the ethyl acetate fraction. This is the first report on the anti-inflammatory activity of *S. ferruginea* parasitizing on different host plants, as well as the extensive study concerning its plant metabolites and their correlations with anti-inflammatory property using metabolomics. The results of this study lay the foundation for in-depth study of *S. ferruginea*, and further understanding on the effect of host plants on the bioactivity of the hemiparasitic plant.

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

ANALISIS METABOLOMIK ANTI-INFLAMASI *Scurrula ferruginea* (Jack) Danser PARASIT PADA TIGA TUMBUHAN PERUMAH YANG BERBEZA, DAN PEMAHAMAN MENGENAI KEMUNGKINAN MEKANISME

Oleh

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Pengerusi : Profesor Madya Intan Safinar Ismail, PhD
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Scurrula ferruginea (Jack) Danser dari famili Loranthaceae adalah pokok renek separa-parasitik yang tumbuh di atas tumbuhan dikotiledon. Walaupun telah lama digunakan secara tradisi bagi mengatasi beberapa masalah kesihatan, kajian saintifik ke atas bioaktiviti dan fitokimianya adalah sangat terhad, bahkan masih tiada penyelidikan membabitkan pengaruh hos pada aktiviti kimia serta biologi *S. ferruginea* Tujuan utama kajian ini adalah untuk menilai aktiviti anti-radang *S. ferruginea* dan mekanisme tindakannya, serta mengenalpasti potensi hubungan antara metabolit *S. ferruginea* terhadap anti-radang. Aktiviti anti-radang telah ditaksir melalui kesekatlakuan pengeluaran nitric oksida (NO) di dalam sel makrofag RAW 264.7 teraruh dengan lipopolisakarida (LPS) dan inteferon-γ (IFN-γ). Penyelidikan dimulakan dengan pengeringan secara udara serta sejuk beku pada parasit *S. ferruginea* yang hidup di atas *Vitex negundo*. Dapatan menunjukkan sejuk beku adalah kaedah yang terbaik bagi keputusan aktiviti anti-radang. Kemudian, kajian diteruskan bagi meneliti kemampuan anti-radang pada daun dan batang *S. ferruginea* yang menjadi parasit ke atas tiga jenis hos yang berbeza.. Keputusan menunjukkan batang parasit *S. ferruginea* di atas *Tecoma stans* dan *Vitex negundo* yang telah disejuk-beku mempunyai aktiviti anti-radang yang lebih tinggi berbanding sampel daun. Nilai IC_{50} bagi keduanya ialah 114.47 ± 2.96 dan 118.87 ± 2.31µg/mL Kemudian, mekanisme anti-radang telah didalami melalui utusan asid ribonuklik (mRNA) dan ekspresi protin bagi sitokin-sitokin radang, termasuk nitrik oksida induksi sintes (*i*NOS), siklooksigenes-2 (COX-2), interlukin (IL), dan faktor tumor nekrosis (TNF). Teknik transkriptes balikan dan kuantitatif waktu nyata tindak balas rantaian polimeres (RT-PCR dan qPCR) juga telah digunakan. Batang *S. ferruginea* berhoskan *Tecoma stans* mempunyai kebolehan anti-radang melalui penekanan ekspresi *i*NOS dan IL-1β mRNA, serta kesekatlakuan pengeluaran protin terhadap IL-1β, IL-6, IL-10, dan TNF-α disamping kesekatlakuan rembesan NO. Seterusnya, perbezaan metabolit diteliti menggunakan proton resonans magnetik nuklear (^{1}H NMR) dan kromatografi cecair

dengan spektroskopi jisim (LC-MS) gabungan dengan analisis data multivariat (MVDA). Pendekatan metabolomik telah digunapakai bagi mengkaji variasi metabolit serta menghubungkait aktiviti anti-radang Analisa Komponen Prinsipal (PCA) dengan jelas menunjukkan diskriminasi antara bahagian tumbuhan dan pokok hos yang berbeza berdasarkan metabolit yang dikenalpasti. Kuersitrin, 4''-*O*-asetilkuersitrin, katekina, asid galik dan asid klorogenik, serta alanina, valina, leusin, isoleusin, asid malik, asid suksinik, asid sitrik dan asid asetik adalah lebih tinggi didalam ekstrak daun. Sementara treonina, histidin, asid fumarik dan kolin kebanyakannya dijumpai pada batang. Batang *S. ferruginea-T. stans* juga memiliki kuersitrin, 4''-*O*-asetilkuersitrin, katekin, valina, leusin, isoleusin, asid fumarik, asid galik, asid klorogenik, serta sukrosa yang tinggi dibandingkan dengan batang *S. ferruginea-V. negundo* and *S. ferruginea-M. minutum.* Pemerhatian korelasi dari model regresi Kuasa Dua Terpencil Separa (PLS) mencadangkan bioaktiviti bagi anti-radang berkemungkinan berkaitan dengan kehadiran kolin, isolusin, katekin, lusin, dan asid klorogenik, serta beberapa metabolit yang tidak dikenalpasti seperti ion berjisim *m/z* pada 369.1157, 401.0851, 431.1327, dan 473.1794. Akhir sekali, aktiviti bioaktif terfraksi dengan kaedah fraksi ekstrak "cecair-cecair" pada batang sejuk beku *S. ferruginea* berhos *T. stans* telah dijalankan. Keputusan mendapati fraksi etil asetat menunjukkan kesekatlakuan NO yang paling tinggi dengan 84. 8 ± 1.45% pada 200 µg/mL. Fraksi klorofom pula menghasilkan aktiviti yang lebih tinggi berbanding *n*-butanol dan fraksi etil asetat pada 50 µg/mL, namun begitu fraksi $CHCl_3$ menunjukkan sitotoksik terhadap sel RAW 264.7. Katekin telah dielusidasikan di dalam fraksi etil asetat. Kajian ini merupakan laporan pertama aktiviti anti-radang parasit *S. ferruginea* pada hos tumbuhan yang berlainan, disamping kajian meluas pada metabolit tumbuhan dan korelasi sifat anti-radang menggunakan metabolomik. Hasil dapatan kajian mempamerkan asas yang mendalam untuk kajian *S. ferruginea* serta pemahamaman yang lebih baik mengenai kesan tumbuhan hos pada bioaktiviti tumbuhan separa-parasitik. .

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

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ANOVA	Analysis of variancea
AP-1	Activating protein-1
ATCC	American Type Culture Collection
CD_3OD	Deuterated methanol
CID	Collision-induced dissociation
COX-2	Cyclooxygenase-2
CV	Cross validation
d	Doublet
dd	Doublet of doublet
DMEM	Dulbecco's modified eagle's medium
DMSO	Dimethyl sulfoxide
D_2O	Deuterium oxide
ESI	Electrospray ionization
FBS	Fetal bovine serum
FT-IR	Fourier Transform Infra-red
GAPDH	Glyceraldehyde-3-phosphate dehydrogenase
GC	Gas chromatography
HMDB	Human Metabolome Database
HPLC	High performance liquid chromatography
HSQC	Heteronuclear single quantum correlation
Hz	Hertz
IC_{50}	Inhibition concentration at 50 percent
IFN-γ	Interferon-γ

*i*NOS	Inducible nitric oxide synthase
IL	Interleukin
LC	Liquid chromatography
LC-MS	Liquid chromatography-mass spectrometry
LOX	Lipoxygenase
LPS	Lipopolysaccharide
m	Multiplet
m/z	Mass-to-charge ratio
MAPK	Mitogen-activated protein kinase
mRNA	Messenger Ribonucleic acid
MS	Mass spectrometry
MTT	3-(4,5-dimethy-2-thiazolyl)-2,5-diphenyl-2-*H*-tetrazolium bromide
MVDA	Multivariate data analysis
NaOD	Sodium deuterium oxide
NF-κB	Nuclear factor kappa beta
NMR	Nuclear magnetic resonance
NO	Nitric oxide
OPLS-DA	Orthogonal partial least squares discriminant analysis
PC	Principal component
PCA	Principal component analysis
PBS	Phosphate buffer saline
PLS	Partial least squares
PLS-DA	Partial least squares discriminant analysis

qPCR	Real time quantitative polymerase chain reactions
QTOF	Quadrupole–time of flight
RT-PCR	Reverse transcriptase polymerase chain reactions
s	Singlet
SD	Standard deviation
t	Triplet
TIC	Total ion chromatogram
TNF	Tumor necrosis factor
TSP	Trimethylsilylpropionic acid-*d*4 sodium salt
UHPLC-QTOF-MS	Ultra-high performance liquid chromatography tandem quadrupole time of flight mass spectrometry
UPM	Universiti Putra Malaysia
VIP	Variable importance in projections
δ	Chemical shift
1D	One-dimensional
^{1}H	Proton
2D	Two-dimensional
^{13}C	Carbon-13

CHAPTER 1

INTRODUCTION

1.1 Background

Inflammation, as a part of the body's innate immunity, belongs to one of the natural self-defense systems (Hong et al., 2015). It is a characteristically protective mechanism to guard the body as well as to facilitate the recovery process, which is sparked in response to noxious stimuli, trauma or infection (Nwaehujor et al., 2014). Inflammatory responses no matter acute or chronic play a crucial role to retain immune homeostasis (Hossen et al., 2015). Albeit reacting as a normal response, an inflammatory process is known in contributing and promoting numerous serious diseases such as cancers, stroke, diabetes, cardiovascular diseases, rheumatoid arthritis, and bronchitis (Heo et al., 2010). The disease process could be caused and aggravated by acutely and extremely induced, or chronically persistent inflammatory responses due to the complex mediators and events involved in the inflammatory reactions. Consequently, it is emphasized that regulating the inflammatory process is essential in preventing such illnesses (Yang et al., 2015).

Normally, the inflammatory reactions are controlled and regulated by a series of cytokines and mediators involved in the process of the pro-inflammatory proteins down-regulations and the anti-inflammatory proteins up-regulations to consequent inflammatory restoration (Kim et al., 2008). In inflammation, the role of macrophages has been highlighted wherein in practice the inflammatory cellular model of RAW264.7 murine macrophage cell lines induced by lipopolysaccharide (LPS) is extensively used for the anti-inflammatory study of drugs, medicinal plant ingredient, and herbs (Yoon et al., 2009; Tan et al., 2015; Qin et al., 2010; Kim et al., 2008). The inflammation-related mediators and cytokines (such as nitric oxide, interleukin, interferon, tumor necrosis factor) are frequently chosen as parameters to evaluate and explain the anti-inflammatory effect of experimental samples. Inhibition of pro-inflammatory mediators and cytokines following induction of macrophages is particularly regarded as the key target in the treatment of inflammatory ailments (Yang et al., 2015; Hong et al., 2015). Besides LPS, a number of immunological stimuli, such as interferon-γ (IFN-γ), interleukin-1, and tumor necrosis factor-α can also cause the expression of some inflammatory cytokines in macrophages (Chiou et al., 2000). IFN-γ, originally called macrophage-activating factor, is one of the most potent activators of the nitric oxide synthase (*i*NOS) gene in murine macrophages (Kamijo et al., 1994). In triggering nitric oxide (NO) production, maximal stimulation of *i*NOS transcription requires "priming" and "triggering" stimuli (Schroder et al., 2004). LPS alone can trigger macrophages to a limited extent, while IFN-γ may prime the macrophages for more rapid and maximal response to LPS by promoting ligand-receptor interactions as well as a downstream signaling mechanism (Lowenstein et al., 1993; Schroder et al., 2004).

In recent years, focus on the use of the traditional herb as an alternative treatment has been revived all over the world. The study on natural product bioactivity is one of the major researches in drug discovery including for the potential anti-inflammatory substances. *Scurrula ferruginea* (Jack) Danser (synonym *Loranthus ferrugineus* Roxb.) of Loranthaceae family is a hemi-parasitic shrub that grows on a dicotyledonous tree and attaches itself to the host tree by modified roots named haustorium (Barlow, 1991). *Scurrula ferruginea* natural habitat is around Asian countries including Malaysia, China, Cambodia, Indonesia, Laos, Burma, the Philippines, Thailand, Vietnam, Sumatra, and India, as well as Australia and New Zealand (Wiart, 2012; Barlow, 1991). It is commonly known as Dedalu or Delalu Api in Malaysia, and 'Xiu mao li guo ji sheng' or 'Dian nan ji sheng' in China. In folk medicine, *S. ferruginea* is used in Malaysia to address for skin disorder, inflammation, and ulcer, as well as postpartum protective remedy and to treat malaria, wounds, and snakebite (Herbal Medicine Research Centre & Institute for Medical Research, 2002). As 'Chinese Material Medica' recorded, it is used to cure rheumatism, stroke, hemiplegia, and pain in the waist and knee. With respect to therapeutic effects, *S. ferruginea* is still in the exploration stage. For the past few years, besides its reputable usage as antioxidant, antiviral, antibacterial, and anticancer agent (Ameer et al., 2015; Lohézic-Le Dévéhat et al., 2002a; Marvibaigi et al., 2014), *S. ferruginea* has also shown remarkable activities for hypertension, cardiovascular and gastrointestinal disorders (Ameer et al., 2009a, 2009b, 2009c; 2010a, 2010b, 2010c). This plant is also applied for gerontology including as daily health protection and enhancement of memory (Ameer et al., 2015; Lim et al., 2016). Most members of *Loranthaceae* family, world widely known as mistletoes, have been intensively studied and long been widespread used in medicine. However, *S. ferruginea* has, despite its ethnomedicinal use, only been studied very limited.

It should not be a neglected issue that growth of a hemiparasitic plant might be influenced by its host nature since it can survive through its own photosynthesis abilities but obtain nutrients and water from the host via its well-developed sophisticated system (Lim et al., 2016; Moghadamtousi et al., 2014). Although the mechanisms behind the processes are sparsely documented, some evidence suggested that the host plants might affect hemiparasite performance. It was reported that the types and levels of phytoconstituents and their biological activities of the mistletoe vary significantly due to the parasitizing on the different host species (Le et al., 2016a, 2016b; Vicaş et al, 2011).

The metabolites and biological activities of plant materials can have significant variation at the different plant parts (Marvibaigi et al., 2014; Ayouni et al., 2016), and also be substantially affected by the post-harvest processing, for example the drying method used. Drying treatment is a common method at the preservation of plant materials, which inhibits enzymatic degradation, hydrolysis and limits microbial growth by removing moisture contained in the plant tissue (Ratti, 2001). Different methods of drying have been developed and each method has its own characteristics (Ratti, 2001). Considering the sun or air-drying, water is commonly removed by evaporation. But, in the case of freeze-drying, botanical materials are first frozen and then water is removed by sublimation (Lin et al., 2012). There are various studies

reported regarding the influences of different drying methods on the level of the plant metabolites (Abascal et al., 2005; Borchani et al., 2011; Sogi et al., 2013). Therefore, depending on the plant samples, the effect of different drying methods should be evaluated and optimized.

To use herbs for health care or therapy, the most conventional approach is to drink directly their water decoction. Water is one of the safest solvents in pharmacological research of *in vivo* and *in vitro*. Furthermore, an extensive range of plant components can be extracted into water. However, it also makes it difficult to discover specific active compounds. Alternatively, liquid-liquid partitioning can be carried out to improve the ascertainment of valuable metabolites (Kim et al., 2012; Wolfender et al., 2013). An aqueous crude extract of plant samples is successively partitioned with different organic solvents to obtain the different polar fractions, respectively. This type of approach, in particular the bioactivity-guided fractionation procedure, is used for classical phytochemical investigation until a pure active component is attained (Wolfender et al., 2013; Mothana et al., 2012).

As a holistic, comprehensive, and non-biased analytical approach in the identification and quantitation of metabolites, metabolomic techniques are rapidly developing into a prominent choice in many research aspects including biological and phytochemical studies. Plant metabolomics could provide information even at the most 'functional' level that can be used to monitor significant metabolite variations. Hence, this approach is suitable to evaluate the metabolite variety in certain plant species, investigate chemical differentiation in certain environments, or quantify the bioactive metabolites for medicinal plants (Wolfender et al., 2013). Integrating with various analytical platforms such as nuclear magnetic resonance (NMR) and liquid chromatography-mass spectrometry (LC-MS), comprehensive metabolic fingerprints could be achieved. Chemical variations of some secondary metabolites between plant extracts without chromatographic purification is possibly observed through metabolomics by means of the multivariate data analysis (MVDA) (Georgiev et al., 2011).

1.2 Problem Statements

The high prevalence of inflammation problem leads to the imperious demands to find out alternative drugs from natural resources replacing the synthetic ones. Despite a long time traditional uses in treating some disorders, there is very little scientific evidence on the ethnomedicinal use of *S. ferruginea*, specifically on its use for conditions related to inflammation. Additionally, very little is known about the chemistry of the plant and likewise, very little is known about the bioactive constituents. Moreover, there are many claims but a paucity of scientific evidence on the uses of *S. ferruginea* from different hosts in treating different diseases. There is also no research concerning how does the host plant affects (if any) the chemistry and bioactivity of *S. ferruginea*.

1.3 Research Objectives

1.3.1 General Objective

In consideration of the aforementioned problems, the present study was designed and performed with the general objective to investigate the possible anti-inflammatory activity of *S. ferruginea* harvested from three different host plants. Furthermore, with the aid of metabolomics, this study is aimed to interrogate the exogenic influence of different host plants on anti-inflammatory activity of *S. ferruginea*, towards further understanding the effect of host plants on the bioactivity of this hemi-parasitic plant.

1.3.2 Specific Objectives

The current study was carried out to solve the issues main in four aspects, which included anti-inflammatory activity evaluation, anti-inflammatory mechanism study, phytochemical investigation, and relationship analysis between metabolites and bioactivity of *S. ferruginea*. To address the issues, several specific objectives were proposed. The first part of the study aimed to investigate the anti-inflammatory activity of aqueous extracts of *S. ferruginea* parasitizing on three host plants via nitric oxide (NO) inhibitory experiment *in vitro*. The optimization of the drying method was firstly executed by comparing two types of methods, which were air- and freeze-dry. The dried leaves and stems of *S. ferruginea* using optimized drying method were then extracted with deionized water to evaluate the effect of different plant parts from different host plants on the anti-inflammatory activity. In the second part, the most active aqueous extract of *S. ferruginea* part from the most active host plant was chosen to decipher the possible anti-inflammatory mechanism through the inhibition of inflammatory cytokines at messenger Ribonucleic acid (mRNA) and protein levels expression. Besides, the aqueous extract with the highest activity was also selected to do bioactivity-guided fractionation by liquid-liquid extraction in order to discover easily the bioactive compounds from bioactive fractions. Additionally, the study was carried out regarding *S. ferruginea* phytochemical components and their correlations with anti-inflammatory activity using NMR- and LCMS-based metabolomics. The relationship between the metabolite profiles of *S. ferruginea* with its anti-inflammatory potency was determined. Furthermore the structures of the phytochemical markers were elucidated. In particular, in view of the hemi-parasitic characteristic of *S. ferruginea*, the exogenic effects of the host plants on its anti-inflammatory property were distinguished.

The five specific objectives are presented as follow:

1. To evaluate the effect of different drying methods on the anti-inflammatory activity of *S. ferruginea.*
2. To compare the anti-inflammatory abilities of leaves and stems of *S. ferruginea* collected from three different host plants (*i.e. Vitex negundo* L., *Micromelum minutum* (G. Forst.) Wight & Arn., and *Tecoma stans* (L.) Juss ex HBK).

3. To decipher the possible mechanism of the anti-inflammatory property of the most active *S. ferruginea* sample.
4. To attempt to seek for the phytochemical compounds of *S. ferruginea* with anti-inflammatory property by bioactivity-guided liquid-liquid fractionation procedure.
5. To evaluate the influence of different extracts of *S. ferruginea* on phytochemical constituents and the correlation with anti-inflammatory activity, as well as to elucidate the chemical markers using ^{1}H NMR- and LCMS - based metabolomics.

REFERENCES

Aardema, M. J. & MacGregor, J. T. (2002). Toxicology and genetic toxicology in the new era of "toxicogenomics": impact of "-omics" technologies. *Mutation Research*, 499, 13-25

Abascal, K., Ganora, L., & Yarnell, E. (2005). The effect of freeze-drying and its implications for botanical medicine: A Review. *Phytotherapy Research*, *19*(September 2004), 655–660. http://doi.org/10.1002/ptr.1651

Abdul-Hamid, N. A., Abas, F., Ismail, I. S., Shaari, K., & Lajis, N. H. (2015). Influence of different drying treatments and extraction solvents on the metabolite profile and nitric oxide inhibitory activity of Ajwa Dates. *Journal of Food Science*, *80*(11), H2603–H2611. http://doi.org/ 10.1111/1750-3841.13084

Abramson, S. B., Amin, A. R., Clancy, R. M., & Attur, M. (2001). The role of nitric oxide in tissue destruction. Best practice & research. *Clinical rheumatology 15*, 831-845.

Alam, M. I. & Gomes, A. (2003). Snake venom neutralization by Indian medicinal plants (*Vitex negundo* and *Emblica officinalis*) root extracts. *Journal of Ethnopharmacology 86*, 75-80. http://doi.org/10.1016/ S0378-8741 (03)00049-7

Ameer, O. Z., Salman, I. M., Najim, H. S., Abdullah, G. Z., Abdulkarim, M. F., Yam, M. F., … Asmawi, M. Z. (2010b). *In vitro* pharmacodynamic profile of *Loranthus ferrugineus*: Evidence for noncompetitive antagonism of norepinephrine-induced vascular contraction. *JAMS Journal of Acupuncture and Meridian Studies*, *3*(4), 272–282. http://doi.org/ 10.1016/S2005-2901(10)60048-9

Ameer, O. Z., Salman, I. M., Quek, K. J., & Asmawi, M. Z. (2015). *Loranthus ferrugineus*: a Mistletoe from traditional uses to laboratory bench. *Journal of Pharmacopuncture*, *18*(1), 7–18. http://doi.org/10.3831/ KPI.2015.18.001

Ameer, O. Z., Salman, I. M., Siddiqui, M. J. A., Yam, M. F., Sriramaneni, R. N., Mohamed, A. J., … Asmawi, M. Z. (2010a). Pharmacological mechanisms underlying the vascular activities of *Loranthus ferrugineus* Roxb. in rat thoracic aorta. *Journal of Ethnopharmacology*, *127*(1), 19–25. http://doi.org/10.1016/j.jep.2009.09.057

Ameer, O. Z., Salman, I. M., Siddiqui, M. J. A., Yam, M. F., Sriramaneni, R. N., Sadikun, A., … Asmawi, M. Z. (2009a). Characterization of the possible mechanisms underlying the hypotensive and spasmogenic effects of *Loranthus ferrugineus* methanolic extract. *American Journal of Chinese Medicine*, *37*(5), 991–1008. http://doi.org/10.1142/S0192415X09007405

Ameer, O. Z., Salman, I. M., Siddiqui, M. J. A., Yam, M. F., Sriramaneni, R. N., Sadikun, A., … Asmawi, M. Z. (2009b). *In vitro* cholinomimetic effect of *Loranthus ferrugineus* in isolated Guinea pig ileum. *JAMS Journal of Acupuncture and Meridian Studies*, *2*(4), 288–293. http://doi.org/10.1016/S2005-2901(09)60070-4

Ameer, O. Z., Salman, I. M., Siddiqui, M. J. A., Yam, M. F., Sriramaneni, R. N., Sadikun, A., … Asmawi, M. Z. (2010c). Cardiovascular activity of the *n*-butanol fraction of the methanol extract of *Loranthus ferrugineus* Roxb. *Brazilian Journal of Medical and Biological Research*, *43*(2), 186–194. http://doi.org/10.1590/S0100-879X2010005000002

Ameer, O. Z., Salman, I. M., Yam, M. F., Abd Allah, H. H., Abdulla, M. H., Shah, A. M., … Asmawi, M. Z. (2009c). Vasorelaxant properties of *Loranthus ferrugineus* Roxb. methanolic extract. *International Journal of Pharmacology*, *5*(1), 44–50. http://doi.org/10.3923/ijp.2009.44.50

Artanti, N., Firmansyah, T., & Darmawan, A. (2012). Bioactivities evaluation of indonesian mistletoes (*Dendrophthoe pentandra* (L.) Miq.) leaves extracts. *Journal of Applied Pharmaceutical Science*, *2*(1), 24–27.

Atsumi, T., Murakami, Y., Shibuya, K., Tonosaki, K., and Fujisawa, S. (2005). Induction of cytotoxicity and apoptosis and inhibition of cyclooxygenase-2 gene expression, by Curcumin and its analog, *α*-diisoeugenol. *Anticancer Research*, *25*(5), 4029-4036.

Ayouni, K., Berboucha-Rahmani, M., Kim, H. K., Atmani, D., Verpoorte, R., and Choi, Y. H. (2016). Metabolomic tool to identify antioxidant compounds of *Fraxinus angustifolia* leaf and stem bark extracts. *Industrial Crops and Products*, *88*, 65-77. http://dx. doi.org/10.1016/j.indcrop. 2016.01.001.

Azhar, U. H., Malik, A., Anis, I., Khan, S.B., Ahmed, E., Ahmed, Z., Nawaz, S.A. and I., C.M. (2004). Enzyme inhibiting lignans from *Vitex negundo*. *Chemical and Pharmaceutical Bulletin. 52,* 1269-1272.

Barlow, B. A. (1991). Provisional key to the genera of Loranthaceae and Viscaceae of the flora Malesiana region. *Flora Malesiana Bull*, *10*, 335–338.

Bennett, R. N., & Wallsgrove, R. M. (1994). *Tansley review no. 72. Secondary metabolites in plant defence mechanisms*, (72), 617–633.

Bilia, A.R., Ciampi, L., Mendez, J., Morelli, I. (1996). Phytochemical investigations of *Licania* genus. Flavonoids from *Licania pyrifolia*. *Pharmaceutica Acta Helvetiae*, 71, 199–204.

Borchani, C., Besbes, S., Masmoudi, M., Blecker, C., Paquot, M., & Attia, H. (2011). Effect of drying methods on physico-chemical and antioxidant properties of date fibre concentrates. *Food Chemistry*, *125*(4), 1194–1201. http://doi.org/10.1016/j.foodchem.2010.10.030

Brindle, J. T, Antti, H., Holmes, E., Tranter, J. K., Nicholson, J. K., … Grainger, D. J. (2002). Rapid and noninvasive diagnosis of the presence and severity of coronary heart disease using 1H-NMR-based metabonomics. *Nature Medicine, 8*(12), 1439-1445.

Brouet I. & Ohshima H. (1995). Curcumin, an anti-tumor promoter and anti-inflammatory agent, inhibits induction of nitric oxide synthase in activated macrophages. *Biochemical and Biophysical Research Communications, 206*(2), 533–540.

Calixto, J. B., Campos, M. M., Otuki, M. F., & Santos, A. R. S. (2004). Anti-inflammatory compounds of plant origin. Part II. Modulation of pro-inflammatory cytokines, chemokines and adhesion molecules. *Planta Medica, 70*(2), 93–103. http://doi.org/10.1055/s-2004-815483

Chandramu, C., Manohar, R. D., Krupadanam, D. G. I., & Dashavantha, R. V. (2003). Isolation, characterization and biological activity of betulinic acid and ursolic acid from *Vitex negundo* L. *Phytotherapy Research, 17,* 129-134

Chen, C. Y., Peng, W. H., Tsai, K. D., & Hsu, S. L. (2007). Luteolin suppresses inflammation-associated gene expression by blocking NF-κB and AP-1 activation pathway in mouse alveolar macrophages. *Life Sciences, 81*(23–24), 1602–1614. http://doi.org/10.1016/j.lfs.2007.09.028

Chen, Y. C., Shen, S. C., Chen, L. G., Lee, T. J. F., Yang, L. L. (2001). Wogonin, baicalin, and baicalein inhibition of inducible nitric oxide synthase and cyclooxygenase-2 gene expressions induced by nitric oxide synthase inhibitors and lipopolysaccharide. *Biochemical Pharmacology, 61,* 1417-1427.

Chen, D., Nie, M., Fan, M. W., & Bian, Z. (2008). Anti-inflammatory activity of curcumin in macrophages stimulated by lipopolysaccharides from *Porphyromonas gingivalis*. *Pharmacology, 82*(4), 264–269. http://doi.org/ 10.1159/000161127

Chinese Materia Medica Editorial Board, National Administration of Traditional Chinese Medicine. (1999). *Chinese Materia Medica*. Shanghai Science and Technology Press, Shanghai, China.

Chiou, W. F., Chen, C. F., & Lin, J. J. (2000). Mechanisms of suppression of inducible nitric oxide synthase (*i*NOS) expression in RAW 264.7 cells by andrographolide. *British Journal of Pharmacology, 129*(8), 1553–1560. http://doi.org/ 10.1038/sj.bjp.0703191

Chiou, W. F., Peng, C. H., Chen, C. F., Chou, C. J. (2003). Anti-inflammatory properties of piperlactam S: modulation of complement 5a-induced chemotaxis and inflammatory cytokines production in macrophages. *Planta Medica, 69,* 9 - 14.

Chiu, J. H., Lay, I. S., Su, M. Y., Chiu, H. L., Chiu, S. N., Lui, W. Y., Wu, C. W. (2002).Tumor necrosis factor-producing activity of wogonin in RAW 264.7 murine macrophage cell line. *Planta Medica, 68,*1036-1039.

Cho, J. Y., Kim, A. R., Park, M. H. (2001). Lignans from the rhizomes of *Coptis japonica* differentially act as anti-inflammatory principles. *Planta Medica, 67,* 312-316.

Cho, S. K., Yang, S. O., Kim, S. H., Kim, H., Ko, J. S., Riu, K. Z., … Choi, H. K. (2009). Classification and prediction of free-radical scavenging activities of dangyuja (*Citrus grandis* Osbeck) fruit extracts using 1H NMR spectroscopy and multivariate statistical analysis. *Journal of Pharmaceutical and Biomedical Analysis, 49*(2), 567–571. http://doi.org/ 10.1016/j.jpba.2008.11.037

Connelly, L., Palacios-Callender, M., Ameixa, C., Moncada, S., & Hobbs, A. J. (2001). Biphasic regulation of NF- κB activity underlies the pro- and anti-inflammatory actions of nitric oxide. *The Journal of Immunology*, *166*(6), 3873–3881. http://doi.org/10.4049/jimmunol.166.6.3873

Costantino, L., Raimondi, L., Pirisino, R., Brunetti, T., Pessotto, P., Giannessi, F., … El-Abady, S. A. (2003). Isolation and pharmacological activities of the *Tecoma stans* alkaloids. *IL Farmaco, 58,* 781-785. http://doi.org/ 10.1016/S0014-827X(03)00133-2

Dash, S., Das, C., Sahoo, D. C., & Sahoo, A. C. (2011). Phytochemical composition, anti-inflamatory and analgesic activities of *Tecoma stans Linn*. (Bignoniaceae). *Nature of Pharmaceutical Technology* 1(2): 5-8.

Davis, A. L., Cai, Y., Davies, A. P., Lewis, J. R. ^{1}H and ^{13}C NMR assignments of some green tea polyphenols. *Magnetic Resonance in Chemistry.* 1996; 34:887-890.

Dharmasiri, M. G., Jayakody, J. R. A. C., Galhena, G., Liyanage, S. S. P. & Ratnasooriya, W. D. (2003). Anti-inflammatory and analgesic activities of mature fresh leaves of *Vitex negundo*. *Journal of Ethnopharmacology. 87,* 199-206.

Diaz, F., Chavez, D., Lee, D., Mi, Q., Chai, H. B., Tan, G. T. … Kinghorn, A. D. (2003). Cytotoxic flavone analogues of Vitexicarpin, a constituent of the leaves of *Vitex negundo. Journal of Natural Products, 66,* 865-867.

Duan, H., Takaishi, Y., Momota, H., Ohmoto, Y., Taki, T., Jia, Y., Li, D. (2001). Immunosuppressive sesquiterpene alkaloids from *Tripterygium wilfordii*. *Journal of Natural Products, 64,* 582-587.

Elsyana, V., Bintang, M., & Priosoeryanto, B. P. (2016). Cytotoxicity and antiproliferative activity assay of clove mistletoe (*Dendrophthoe pentandra* (L.) Miq.) leaves extracts. *Advances in Pharmacological Sciences*, *2016*. http://doi.org/10.1155/2016/3242698

Eming, S.A., Krieg, T., & Davidson, J.M. (2007). Inflammation in wound repair: molecular and cellular mechanisms. *The Journal of investigative dermatology 127*, 514-525.

Fiehn, O. (2001). Combining genomics, metabolome analysis, and biochemical modeling to understand metabolic networks. *Comparative and functional genomics*, 2, 155-168.

Fiehn, O. (2003). Metabolic networks of *Cucurbita maxima* phloem. *Phytochemistry, 62* (6), 875-866.

Georgiev, M. I., Ali, K., Alipieva, K., Verpoorte, R., & Choi, Y. H. (2011). Metabolic differentiations and classification of Verbascum species by NMR-based metabolomics. *Phytochemistry*, *72*(16), 2045–2051. http://doi.org/10.1016/j.phytochem.2011.07.005

Govindappa, M., Sadananda, T.S., Channabasava, R., Jeevitha, M.K., Pooja, K.S. & Vinay, B. Raghavendra. (2011). Antimicrobial, antioxidant activity and phytochemical screening of *Tecoma Stans* (L.) Juss. ex Kunth. *Journal of Phytology*, *3*(3), 68-76

Guimarães, M. R., Leite, F. R. M., Spolidorio, L. C., Kirkwood, K. L., & Rossa, C. (2013). Curcumin abrogates LPS-induced proinflammatory cytokines in RAW 264.7 macrophages. Evidence for novel mechanisms involving SOCS-1, -3 and p38 MAPK. *Archives of Oral Biology*, *58*(10), 1309–1317. http://doi.org/10.1016/j.archoralbio.2013.07.005

Haddad, J. J. (2002). Cytokines and related receptor-mediated signaling pathways. *Biochemical and Biophysical Research Communications*, *297*(4), 700–713. http://doi.org/10.1016/S0006-291X(02)02287-8

Haskó, G., Szabó, C., Németh, Z. H., Kvetan, V., S. M. P. and E. S. V. (1996). Adenosine receptor agonists differentially regulate IL-10, TNF-alpha, and nitric oxide production in RAW 264.7 macrophages and in endotoxemic mice. *The Journal of Immunology*, *157*(10), 4634–4640.

Haskó, G., Németh, Z. H., Szabó, C., Zsilla, G., Salzman, A. L., and Vizi, E. S. (1998). Isoproterenol inhibits IL-10, TNF-α, and nitric oxide production in RAW 264.7 macrophages. *Brain Research Bulletin 45*(2):183-187.

Henry, C. M. (2002). New ome in town. The 'metabonome' provides real-world information about drug toxicity, gene function. *Chemical & engineering news*, *80*(48), 66.

Heo, S. J., Yoon, W. J., Kim, K. N., Ahn, G. N., Kang, S. M., Kang, D. H., … Jeon, Y. J. (2010). Evaluation of anti-inflammatory effect of fucoxanthin isolated from brown algae in lipopolysaccharide-stimulated RAW 264.7 macrophages. *Food and Chemical Toxicology*, *48*(8–9), 2045–2051. http://doi.org/10.1016/j.fct.2010.05.003

Herbal Medicine Research Centre & Institute for Medical Research. (2002). *Compendium of medicinal plants used in Malaysia* (volume 2). Kuala Lumpur, Malaysia.

Hong, J. M., Kwon, O. K., Shin, I. S., Song, H. H., Shin, N. R., Jeon, C. M., … Ahn, K. S. (2015). Anti-inflammatory activities of *Physalis alkekengi* var. *franchetii* extract through the inhibition of MMP-9 and AP-1 activation. *Immunobiology*, *220*(1), 1–9. http://doi.org/10.1016/j.imbio.2014.10.004

Hossen, M. J., Baek, K. S., Kim, E., Yang, W. S., Jeong, D., Kim, J. H., … Cho, J. Y. (2015). *In vivo* and *in vitro* anti-inflammatory activities of *Persicaria chinensis* methanolic extract targeting Src/Syk/NF-*κ*B. *Journal of Ethnopharmacology*. http://doi.org/10.1016/j.jep.2014.10.064

Hwang, S. J., Kim, Y. W., Park, Y., Lee, H. J., Kim, K. W. (2014). Anti-inflammatory effects of chlorogenic acid in lipopolysaccharide-stimulated RAW 264.7 cells. *Inflammation Research*, 63, 81-90. http://doi.org/10.1007/s00011-013-0674-4

Ito, C., Otsuka, T., Ruangrungsi, N., & Furukawa, H. (2000). Chenmical constituents of *Micromelum minutum*. isolation and structural elucidation of new coumarins. *Chemical & Pharmaceutical Bulletin*, *48*(3), 334–338.

Jun, C. D., Choi, B. M., H. M. K. and H. T. C. (1995). Involvement of protein kinase C during taxol-induced activation of murine peritoneal macrophages. *The Journal of Immunology*, *154*(12), 6541–6547.

Jung H. A., Park J. C., Chung H. Y., Kim J., Choi J. S. (1999). Antioxidant flavonoids and chlorogenic acid from the leaves of *Eriobotrya japonica*. *Archives of Pharmacal Research, 22* (2), 213–218.

Jurenka, J. S., & Ascp, M. T. (2009). Anti-inflammatory properties of Curcumin , a major constituent of curcuma longa : A Review of preclinical and clinical research. *Alternative Medicine Review*, *14*(2), 141–153.

Kameshwaran, S., Suresh, V., Arunachalam, G., Frank, P. R., & Manikandan, V. (2012). Evaluation of antinociceptive and anti-inflammatory potential of flower extract *Tecoma stans*. *Indian Journal of Pharmacology, 44*(4), 543–544. http://doi.org/10.4103/0253-7613.99352

Kamijo, R., Harada, H., Matsuyama, T., Bosland, M., Gerecitano, J., … Vilcek, J. (1994). Requirement for transcription factor IRF-1 in NO synthase induction in macrophages. *Science, 263*(5153), 1612–1615.

Karin, M., Lawrence, T., & Nizet, V. (2006). Innate immunity gone awry: linking microbial infections to chronic inflammation and cancer. *Cell 124*, 823-835.

Keun, H. C., Ebbels, T. M. D., Antti, H., Bollard, M. E., Beckonert, O., … Nicholson, J. K. (2002). Analytical reproducibility in 1H NMR-based metabonomic urinalysis. *Chemical Research in Toxicology,15* (11), 1380-1386.

Khokra, S. L., Prakash, O., Jain, S., Aneja, K. R., & Dhingra, Y. (2008). Essential oil composition and antibacterial studies of *Vitex negundo* Linn. extracts. *Indian Journal of Pharmaceutical Sciences, 70* (4), 522-526.

Khoo, L. W., Mediani, A., Zolkeflee, N. K. Z., Leong, S. W., Ismail, I. S., Khatib, A., … Abas, F. (2015). Phytochemical diversity of *Clinacanthus nutans* extracts and their bioactivity correlations elucidated by NMR based metabolomics. *Phytochemistry Letters*, *14*, 123–133. http://doi.org/10.1016/j.phytol.2015.09.015

Kim, H.P., Son, K.H., Chang, H.W., Kang, S.S. (2004). Anti-inflammatory plant flavonoids and cellular action mechanisms. *Journal of Pharmacological Sciences. 96*, 229–245. http://doi.org/10.1254/jphs.CRJ04003X

Kim, J. Y., Park, S. J., Yun, K. J., Cho, Y. W., Park, H. J., & Lee, K. T. (2008). Isoliquiritigenin isolated from the roots of *Glycyrrhiza uralensis* inhibits LPS-induced *i*NOS and COX-2 expression via the attenuation of NF-*κ*B in RAW 264.7 macrophages. *European Journal of Pharmacology, 584*(1), 175–184. http://doi.org/10.1016/j.ejphar.2008.01.032

Kim, Y. K., Kim, R. G., Park, S. J., Ha, J. H., Choi, J. W., Park, H. J., Lee, K. T. (2002). *In vitro* anti-inflammatory activity of kalopanaxsaponin A isolated from *Kalopanax pictus* in murine macrophage RAW 264.7 cells. *Biological & Pharmaceutical Bulletin, 25*, 72 -76.

Korhonen, R., Lahti, A., Kankaanranta, H., & Moilanen, E. (2005). Nitric oxide production and signaling in inflammation. Current drug targets. *Inflammation and allergy 4*, 471-479.

Kracht, M., & Saklatvala, J. (2002). Transcriptional and post-transcriptional control of gene expression in inflammation. *Cytokine*, *20*(3), 91–106. http://doi.org/10.1006/cyto.2002.0895

Kumar, P. P., Kumaravel, S., & Lalitha C. (2003). Screening of antioxidant activity, total phenolics and GC-MS study of *Vitex negundo*. *African Journal of Biochemistry Research, 4*(7), 191-195.

Kwanda, N., Noikotr, K., Sudmoon, R., Tanee, T., & Chaveerach, A. (2013). Medicinal parasitic plants on diverse hosts with their usages and barcodes. *Journal of Natural Medicines*, *67*(3), 438–445. http://doi.org/10.1007/s11418-012-0695-2

Le, Q. V., Tennakoon, K. U., Metali, F., Lim, L. B. L., & Bolin, J. F. (2016a). Host specific variation in photosynthesis of an obligate xylem-tapping mistletoe *Dendrophthoe curvata* in a Bornean heath forest. *Nordic Journal of Botany*, *34*(2), 235–243. http://doi.org/10.1111/njb.00628

Le, Q. V., Tennakoon, K. U., Metali, F., Lim, L. B. L., & Bolin, J. F. (2016b). Ecophysiological responses of mistletoe *Dendrophthoe curvata* (Loranthaceae) to varying environmental parameters. *Journal of Tropical Forest Science*,

28(1), 59–67.

Lee, K.H., Ab Aziz, F.H., Syahida, A., Abas, F., Shaari, K., Israf, D.A., & Lajis, N.H. (2009). Synthesis and biological evaluation of curcumin-like diarylpentanoid analogues for anti-inflammatory, antioxidant and anti- tyrosinase activities. *European journal of medicinal chemistry 44*, 3195-3200.

Lee, S. Y., Abas, F., Sa, I., & Shaari, K. (2016). Metabolite profiling of *Neptunia oleracea* and correlation with antioxidant and α-glucosidase inhibitory activities using 1 H NMR- based metabolomics. *Phytochemistry Letters*, *16*, 23–33. http://doi.org/10.1016/j.phytol.2016.02.014

Lekphrom, R., Kanokmedhakul, S., Kukongviriyapan, V., & Kanokmedhakul, K. (2011). C-7 Oxygenated coumarins from the fruits of *Micromelum minutum*. *Archives of Pharmacal Research*, *34*(4), 527–531. http://doi.org/ 10.1007/s12272-011-0402-y

Li, H. L., Chen, H. L., Li, H., Zhang, K. L., Chen, X. Y., Wang, X. W., … Liu, J. (2005). Regulatory effects of emodin on NF-kappaB activation and inflammatory cytokine expression in RAW 264.7 macrophages. *International Journal of Molecular Medicine*, *16*(1), 41–47.

Liang, G., Zhou, H., Wang, Y., Gurley, E. C., Feng, B., Chen, L., … Li, X. (2009). Inhibition of LPS-induced production of inflammatory factors in the macrophages by mono-carbonyl analogues of curcumin. *Journal of Cellular and Molecular Medicine*, *13*(9 B), 3370–3379. http://doi.org/10.1111/ j.1582-4934.2009.00711.x

Lim, Y. C., Rajabalaya, R., Lee, S. H. F., Tennakoon, K. U., Le, Q. V., Idris, A., … David, S. R. (2016). Parasitic mistletoes of the genera Scurrula and Viscum: From bench to bedside. *Molecules*, *21*(8). http://doi.org/10.3390/ molecules21081048

Lin, L., Lei, F., Sun, D. W., Dong, Y., Yang, B., & Zhao, M. (2012). Thermal inactivation kinetics of *Rabdosia serra* (Maxim.) Hara leaf peroxidase and polyphenol oxidase and comparative evaluation of drying methods on leaf phenolic profile and bioactivities. *Food Chemistry*, *134*(4), 2021–2029. http://doi.org/10.1016/j.foodchem.2012.04.008

Lins, A. P. and Felicio, J. D. (1993). Monoterpene alkaloids from *Tecoma stans*. *Phytochemistry*, *34*(3), 876-878.

Liu, Q. Y., Feng, S., Zhang, Y. H., & Ni, F. (2016). Chemical constituents of *Scurrula parasitica*. *China journal of chinesse materia medica*, *41*(21), 3956–3961. http://doi.org/10.4268/cjcmm.20162112

Liu, Q. Y., Wang, F., Zhang, L., Xie, J. M., Li, P., & Zhang, Y. H. (2015). A Hydroxylated lupeol-based triterpenoid ester isolated from the *Scurrula parasitica* parasitic on Nerium indicum. *Helvetica Chimica Acta*, *98*(5), 627–632. http://doi.org/10.1002/hlca.201400254

Liu, Y. W., Chen, C. C., Tseng, H. P., & Chang, W. C. (2006). Lipopolysaccharide-induced transcriptional activation of interleukin-10 is mediated by MAPK- and NF-κB-induced CCAAT/enhancer-binding protein δ in mouse macrophages. *Cellular Signalling*, *18*(9), 1492–1500. http://doi.org/10.1016/j.cellsig.2005.12.001

Lohézic-Le Dévéhat, F., Bakhtiar, A., Bézivin, C., Amoros, M., & Boustie, J. (2002a). Antiviral and cytotoxic activities of some Indonesian plants. *Fitoterapia*, *73*(5), 400–405. http://doi.org/10.1016/S0367-326X (02)00125-9

Lohézic-Le Dévéhat, F., Tomasi, S., Fontanel, D., & Boustie, J. (2002b). Flavonols from Scurrula ferruginea Danser (Loranthaceae). *Zeitschrift Fur Naturforschung - Section C Journal of Biosciences*, *57*(11–12), 1092–1095. http://doi.org/10.1515/znc-2002-11-1224

Lowenstein, C. J., Alley, E. W., Raval, P., Snowman, A. M., Snyder, S. H., …Murphy, W. J. (1993). Macrophage nitric oxide synthase gene: Two upstream regions mediate induction by interferon γ and lipopolysaccharide. *Proceedings of the National Academy of Sciences of the United States of America*, *90*, 9730-9734. http://doi.org/10.1073/pnas.90.20.9730

Lu, X, Zhao, X. J., Bai, C. M., Zhao, C. X., Lu, G., & Xu, G. W. (2008). LC-MS-based metabonomics analysis. *Journal of chromatography B*, *866*, 64–76. http://doi.org/10.1016/j.jchromb.2007.10.022

Marvibaigi, M., Amini, N., Supriyanto, E., Jamil, S., Majid, F. A. A., & Khangholi, S. (2014). Total phenolic content, antioxidant and antibacterial properties of *Scurrula ferruginea* extracts. *Jurnal Teknologi*, *70*(5), 65–72. http://doi.org/10.11113/jt.v70.3517

Marvibaigi, M., Amini, N., Supriyanto, E., Majid, F. A. A., Jaganathan, S. K., Jamil, S., … Nasiri, R. (2016). Antioxidant activity and ROS-dependent apoptotic effect of *Scurrula ferruginea* (Jack) Danser methanol extract in human breast cancer cell MDA-MB-231. *PLoS ONE*, *11*(7), 1–36. http://doi.org/10.1371/journal.pone.0158942

Marzouk, M., Gamal-Eldeen, A., Mohamed, M., & El-Sayed, M. (2006) Anti-proliferative and antioxidant constituents from *Tecoma stans*. *Zeitschrift fur Naturforschung C, 61*(11-12), 783-791.

Maulidiani, M., Sheikh, B. Y., Mediani, A., Wei, L. S., Ismail, I. S., Abas, F., & Lajis, N. H. (2015). Differentiation of *Nigella sativa* seeds from four different origins and their bioactivity correlations based on NMR-metabolomics approach. *Phytochemistry Letters*, *13*, 308–318. http://doi.org/10.1016/j.phytol.2015.07.012

Mocellin, S., Marincola, F., Rossi, C. R., Nitti, D., & Lise, M. (2004). The multifaceted relationship between IL-10 and adaptive immunity: Putting together the pieces of a puzzle. *Cytokine and Growth Factor Reviews*, *15*(1), 61–76. http://doi.org/10.1016/j.cytogfr.2003.11.001

Moore, K. W., Malefyt, R. D. W., Robert, L., & Garra, A. O. (2001). Interleukin -10 and the interleukin -10 receptor. *Annual Review of Immunology*, *19*, 683–765.

Mothana, R. A. A., Al-Said, M. S., Al-Rehaily, A. J., Thabet, T. M., Awad, N. A., Lalk, M., & Lindequist, U. (2012). Anti-inflammatory, antinociceptive, antipyretic and antioxidant activities and phenolic constituents from *Loranthus regularis* Steud. ex Sprague. *Food Chemistry*, *130*(2), 344–349. http://doi.org/10.1016/j.foodchem.2011.07.048

Mustarichie, R., Warya, S., Saptarini, N. M., & Ramdhani, D. (2015). Total flavonoid content and anti-inflammatory properties of indonesian mistletoes (*Dendrophthoe pentandra* (L.) Miq.) ethanol extract. *World Journal of Pharmaceutical Research*, *4*(4), 287–302.

Nicholson, J. K. & Wilson, I. D. (2003). Understanding 'global' systems biology: Metabonomics and the continuum of metabolism. *Nature Reviews Drug Discovery*, 2, 668-676.

Noreen, Y., Serrano, G., Perera, P., Bohlin, L. (1998). Flavan-3-ols isolated from some medicinal plants inhibiting COX-1 and COX-2 catalysed prostaglandin biosynthesis. *Planta Medica*, 64, 520-524.

Nwaehujor, C. O., Ezeja, M. I., Udeh, N. E., Okoye, D. N., & Udegbunam, R. I. (2014). Anti-inflammatory and antioxidant activities of *Mallotus oppositifolius* (Geisel) methanol leaf extracts. *Arabian Journal of Chemistry*, *7*(5), 805–810. http://doi.org/10.1016/j.arabjc.2012.03.014

Ohashi, K., Winarno, H., Mukai, M., Inoue, M., Prana, M. S., Simanjuntak, P., & Shibuya, H. (2003). Indonesian Medicinal Plants. XXV. Cancer cell invasion inhibitory effects of chemical constituents in the parasitic plant *Scurrula atropurpurea* (Loranthaceae). *Chemical & Pharmaceutical Bulletin*, *51*(3), 343–345.

Okuda, T., Fukushimad, M., & Corporation, P. (1987). Corianin from *Coriaria japonica* A . GRAY , and sesquiterpene lactones from *Loranthus parasiticus* MERR . Used for treatment of schizophrenia. *Chemical & Pharmaceutical Bulletin*, *35*, 182–187.

Osadebe, P. O., Okide, G. B., & Akabogu, I. C. (2004). Study on anti-diabetic activities of crude methanolic extracts of *Loranthus micranthus* (Linn.) sourced from five different host trees. *Journal of Ethnopharmacology*, *95*(2–3), 133–138. http://doi.org/10.1016/j.jep.2004.06.029

Parmely,M. J., W W Zhou, C K Edwards 3rd, D R Borcherding, R. S. and D. C. M. (1993). Adenosine and a related carbocyclic nucleoside analogue selectively inhibit tumor necrosis factor-alpha production and protect mice against endotoxin challenge. *The Journal of Immunology*, *151*(1), 389–396.

Pelander, A., Ojanpera, I., Laks, S., Rasanen, I., & Vuori, E. (2003) Toxicological screening with formula-based metabolite identification by liquid

chromatography/time-of-flight mass spectrometry. *Analytical Chemistry, 75*(21), 5710-5718.

Prasanna, V. L., Lakshman, K., Hegde, M. M., & Bhat, V. (2013). Antinociceptive and anti-inflammatory activity of *Tecoma stans* leaf extracts. *Indian Journal of Research in Pharmacy and Biotechnology*, *1*(April), 156–160.

Qin, J. J., Jin, H. Z., Zhu, J. X., Fu, J. J., Zeng, Q., Cheng, X. R., … Zhang, W. D. (2010). New sesquiterpenes from *Inula japonica* Thunb. with their inhibitory activities against LPS-induced NO production in RAW264.7 macrophages. Tetrahedron, 66(48), 9379–9388. http://doi.org/10.1016/ j.tet.2010.09.091

Qin, Y. R., Mo, T., Liu, X. H., Pan, Z. X., & Ye, M. F. (2010). Effects of Loranthus parasiticus on nutrition metabolism of Osmanthus fragrans and Cinnamomum burmannii. Acta Agriculturea Boreali-Occidentalis Sinica, 4:34.

Rahmani, M., Susidarti, R.A., Ismail, H.B.M., Sukari, M.A., Taufiq-Yap, Y.H., Gwendoline-Ee, C.L., Ali, A.M., Kulip, J. & Waterman P.G. (2003) . Coumarins from Malaysian Micromelum minutum, Phytochemistry, 64, 873-877.

Rahmawati, S. I., Ishimaru, K., Hou, D.-X., & Hayashi, N. (2014). Antioxidant activity and phenolic content of mistletoe extracts following high-temperature batch extraction. Food Science and Technology Research, 20(2), 201–206. http://doi.org/10.3136/fstr.20.201

Ratti, C. (2001). Hot air and freeze-drying of high-value foods: a review. Journal of Food Engineering, 49, 311–319.

Rowley, T. J., McKinstry, A., Greenidge, E., Smith, W., and Flood, P. (2010). Antinociceptive and anti-inflammatory effects of choline in a mouse model of postoperative pain. *British Journal of Anaesthesia*, *105*(2), 201–207. http://doi.org/10.1093/bja/aeq113

Sakunpak, A., Matsunami, K., Otsuka, H., & Panichayupakaranant, P. (2013). Isolation of new monoterpene coumarins from *Micromelum minutum* leaves and their cytotoxic activity against Leishmania major and cancer cells. *Food Chemistry*, *139*(1–4), 458–463. http://doi.org/10.1016/j.foodchem.2013.01.031

Schroder, K., Hertzog, P. J., Ravasi, T., & Hume, D. A. (2004). Interferon-γ: an overview of signals , mechanisms and functions. *Journal of Leukocyte Biology*, *75*(February), 163–189. http://doi.org/10.1189/jlb.0603252

Shakya, A., Singh, G. K., Chatterjee, S. S., Kumar, V. (2014). Role of fumaric acid in anti-inammatory and analgesic activities of a *Fumaria indica* extracts. *Journal of Intercultural Ethnopharmacology*, *3*,173-178. http://doi.org/10.5455/jice.20140912021115

Silverstein, R. M., Webster, F. X., Kiemle, D. J., & Bryce, D. L. (2014). *Spectrometric identification of organic compounds*. John wiley & sons.

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. http://doi.org/10.1016/j.foodchem.2013.05.053

Srinivas, K.K., Rao, S.S., Rao, M.E.B. and Raju, M.B.V. (2001). Chemical constituents of the roots of *Vitex negundo*. *Indian Journal of Pharmaceutical Sciences. 63*, 422-424.

Stitt, M. & Fernie, A. R. (2003). From measurements of metabolites to metabolomics: an 'on the fly' perspective illustrated by recent studies of carbon-nitrogen interactions. *Current Opinion in Biotechnology*, 14: 136-144.

Sumner, L. W., Mendes, M. P., & Dixon, R. A. (2003). Plant metabolomics: large-scale phytochemistry in the functional genomics era. *Phytochemistry*, 62(6), 817-836.

Surh, Y. J., Chun, K. S., Cha, H. H., Han, S. S., Keum, Y. S., Park, K. K., & Lee, S. S. (2001). Molecular mechanisms underlying chemopreventive activities of anti-inflammatory phytochemicals: down-regulation of COX-2 and iNOS through suppression of NF-*κ*B activation. *Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis*, *480–481*, 243–268. http://doi.org/10.1016/S0027-5107(01)00183-X

Susidarti, R.A., Rahmani, M., Ismail, H.B.M., Sukari, M.A., Taufiq-Yap, Y.H., Gwendoline-Ee, C.L., Ali, A.M., Kulip, J. & Waterman P.G. (2006). A new coumarin and triterpenes from Malaysian *Micromelum minutum*, *Natural Product Research, 20*(2), 145-151.

Tan, B. K., Alitheen, N. B., Yeap, S. K., Ali, A. M., & Mawardi, R. (2009). Cytotoxic effect of 2', 3'-epoxy isocapnolactone and 8-hydroxyisocapnolactone-2'3'-diol isolated from *Micromelum minutum* (G. Forst.) Wight and Arn . in human T-lymphocyte leukemia CEM-SS cells. *African Journal of Biotechnology*, *8*(18), 4632-4641.

Tan, W. S., Arulselvan, P., Karthivashan, G., & Fakurazi, S. (2015). *Moringa oleifera* flower extract suppresses the activation of inflammatory mediators in lipopolysaccharide-stimulated RAW 264.7 macrophages via NF-*κ*B pathway. *Mediators of Inflammation, 2015*. http://doi.org/10.1155/2015/ 720171

Tandon, V. R. (2005). Medicinal uses and biological activities of *Vitex negundo*. *Natural Product Radiance*, *4*(3), 162-165.

Tham, C. L., Liew, C. Y., Lam, K. W., Mohamad, A. S., Kim, M. K., Cheah, Y. K., … Israf, D. A. (2010). A synthetic curcuminoid derivative inhibits nitric oxide and proinflammatory cytokine synthesis. *European Journal of Pharmacology*, *628*(1–3), 247–254. http://doi.org/10.1016/j.ejphar.2009. 11.053

Tung, Y. T., Chua, M. T., Wang, S. Y., & Chang, S. T. (2008). Anti-inflammation activities of essential oil and its constituents from indigenous cinnamon (*Cinnamomum osmophloeum*) twigs. *Bioresource Technology, 99*(9), 3908–3913. http://doi.org/10.1016/j.biortech.2007. 07.050

Ueda, H., Yamazaki, C., Yamazaki, M. (2002). Luteolin as an anti-inflammatory and anti-allergic constituent of *Perilla frutescens*. *Biological & Pharmaceutical Bulletin, 25,* 1197-1202.

Verma, S. (2016). Phytochemical and pharmacological review study on *Tecoma Stans* Linn. *Journal of Medicinal Plants Studies, 4*(5), 162-164.

Verpoorte, R., Choi, Y. H., & Kim, H. K. (2007). NMR-based metabolomics at work in phytochemistry. *Phytochemitry Reviews, 6*, 3–14. http://doi.org/10.1007/s11101-006-9031-3

Vicaş, S. I., Rugină, D., & Socaciu, C. (2011). Comparative study about antioxidant activities of *Viscum album* from different host trees, harvested in different seasons. *Journal of Medicinal Plants Research, 5*(11), 2237–2244. Retrieved from http://www.academicjournals.org/JMPR

Wiart, C. (2012). *Medicinal plants of China, Korea, and Japan*. The United States of America: Taylor & Francis Group.

Williamson, E. M. (2001). Synergy and other interactions in phytomedicines. *Phytomedicine*. 8 (5), 401-409.

Wolfender, J., Rudaz, S., Choi, Y. H., & Kim, H. K. (2013). Plant metabolomics: from holistic data to relevant biomarkers. *Current Medicinal Chemistry, 20*, 1056–1090. http://doi.org/10.1111/pce.12002(in press).

Wong, D. Z. H., Kadir, H. A., & Ling, S. K. (2012). Bioassay-guided isolation of neuroprotective compounds from *Loranthus parasiticus* against H_2O_2-induced oxidative damage in NG108-15 cells. *Journal of Ethnopharmacology, 139*(1), 256–264. http://doi.org/10.1016/j.jep.2011. 11.010

Wu, C., and Zhang, G. (1984). Quantitative analysis of sesquiterpene lactone in *Loranthus parasiticus* (L.) Merr. parasitizing on *Coriaria sinica* Maxim and in seeds of *Coriaria sinica* Maxim. *Acta Pharmaceutica Sinica, 19*, 56.

Xie, Q. W., Whisnant, R., Nathan, C. (1993). Promoter of the mouse gene encoding calcium-independent nitric oxide synthase confers inducibility by interferon γ and bacterial lipopolysaccharide. *Journal of Experimental Medicinae, 177*(6), 1779-1784.

Xu, G. W., Lu, X., Yang, S. L. (2007). Recent advances in Metabonomics. *Acta Academiae Medicinae Sinicae, 29*(6), 701-711.

Yang, Y., Lee, J., Rhee, M. H., Yu, T., Baek, K. S., Sung, N. Y., … Cho, J. Y. (2015). Molecular mechanism of protopanaxadiol saponin fraction-mediated anti-

inflammatory actions. *Journal of Ginseng Research*. http://doi.org/ 10.1016/j.jgr.2014.06.002

Yoon, S. Bin, Lee, Y. J., Park, S. K., Kim, H. C., Bae, H., Kim, H. M., … Park, W. (2009). Anti-inflammatory effects of *Scutellaria baicalensis* water extract on LPS-activated RAW 264.7 macrophages. *Journal of Ethnopharmacology*, *125*(2), 286–290. http://doi.org/10.1016/ j.jep.2009.06.027

Zhao, L., Zhang, S. L., Tao, J. Y., Pang, R., Jin, F., Guo, Y. J., … Zheng, G. H. (2008). Preliminary exploration on anti-inflammatory mechanism of Corilagin (beta-1-O-galloyl-3,6-(R)-hexahydroxydiphenoyl-d-glucose) *in vitro*. *International Immunopharmacology*, *8*(7), 1059–1064. http://doi.org/10.1016/j.intimp.2008.03.003

Zorofchian Moghadamtousi, S., Noor Alfarizal Kamarudin, M., Kei Chan, C., Hing Goh, B., & Abdul Kadir, H. (2014). Phytochemistry and Biology of *Loranthus parasiticus* Merr, a Commonly Used Herbal Medicine. *The American Journal of Chinese Medicine*, *42*(1), 23–35. http://doi.org/10.1142/S0192415X14500025

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LIST OF PUBLICATIONS

Journals

Xia Hong, Nurulfiza Mat Isa, Sharida Fakurazi, Intan Safinar Ismail. Phytochemical and anti-inflammatory properties of *Scurrula ferruginea* (Jack) Danser parasitizing on three different host plants elucidated by NMR-based metabolomics. *Phytochemical Analysis*.2020,31(1),15-27. **-Published**

Xia Hong, Mokrish Ajat, Sharida Fakurazi, Akmal Mohd Noor, Intan Safinar Ismail. Anti-inflammatory Evaluation of *Scurrula ferruginea* (Jack) Danser Parasitizing on *Tecoma stans* (L.) H.B.K. in LPS/IFN-γ-Induced RAW 264.7 Macrophages. –**Under review**

Conferences

Xia Hong, Intan Safinar Ismail, Nurulfiza Mat Isa, and Sharida Fakurazi. Anti-inflammatory properties of *Scurrula ferruginea* (Jack) Danser and exogenic effect of its three different host plants elucidated by NMR based metabolomics. International Conference on Natural Products 2018 (Oral Presentation).

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