UNIVERSITI PUTRA MALAYSIA

PHYTOCHEMICAL PROFILE AND ANTI-ALLERGY PROPERTIES OF Clinacanthus nutans (Burm.F) Lindau EXTRACT REVEALED BY ^{1}1H-NMR-BASED METABOLOMICS

KHOO LENG WEI

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By

KHOO LENG WEI

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

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DEDICATION

This thesis is dedicated to my beloved parents

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

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By

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September 2018

Chairman : Associate Professor Faridah Abas, PhD
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Food allergy is an abnormal immunological response arises when a person's immune system wrongly interprets a food as a harmful foreign invader. Although the existing synthetic derived anti-allergy drugs provide effective treatment, the possible side effects on human have drawn the researchers' attention to seek alternatives from nature source. The present study aimed to profile the metabolites and investigate the pharmacological potency of *Clinacanthus nutans*, a local traditional herb and vegetable, in managing food allergy using proton nuclear magnetic resonance (^{1}H-NMR) metabolomics approach. The first and second parts of the study focused on evaluating the effect of different plant parts, dryings, binary extraction solvent system (ethanol: water ratio) and extraction methods on the phytochemical composition and bioactivity of the plant using metabolomics approach. Subsequently, the optimised plant extract was assessed for its acute toxicity effect on Sprague Dawley rats at a single oral dose of 5000 mg/kg body weight (BW) using both conventional (visual behavioural observation, hematological and biochemical tests) and advanced (serum-urinary ^{1}H-NMR metabolomics) toxicity evaluation methods. The optimal plant extract was further evaluated for its anti-allergy effect in a dose dependent manner (125, 500 and 2000 mg/kg BW rat) using *in vivo* ovalbumin induced active systemic anaphylaxis (OVA-ASA) rat model. From the experiment, water-sonication prepared *C. nutans* air dried leaf was determined as the optimal extract as it had the highest TPC (51.44 ± 0.63 mg GAE/g of extract) and exhibited the best NO inhibitory activity (IC_{50}: 190.43 ± 12.26 µg/mL) without adverse effect on the RAW cell viability. The analysis further suggested the potential NO inhibitors in the extract were clinacoside A and B, clinamide A, B and C, phytosterols, lupeol, orientin, isoorientin, valine, alanine, acetic acid, and lactic acid. The acute toxicity test showed that the extract-treated rats behaved normal with no observed morbidity, mortality or apparent signs of toxicity.

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Therefore, the LD_{50} of *C. nutans* extract is likely to be above 5000 mg/kg BW. Consequently, the anti-allergy properties of the optimal *C. nutans* extract was evaluated. A stronger positive correlation of the high dose extract-treated group (2000 mg/kg BW) with the normal group than with the ovalbumin-induced group was observed. The metabolic pathway further suggested the anti-allergy effect of *C. nutans* may be ascribed to its ability in modulating the carbohydrate metabolism, energy metabolism, lipid metabolism, amino acid metabolism and nucleotide metabolism of sensitised rats. This study is the first report on the potency of *C. nutans* as an anti-allergy agent. The finding of this study therefore, paves a new way for future functional food or drug development in mitigating allergy reaction and lays the basis for any metabolomics related study.

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

PROFIL FITOKIMIA DAN CIRI-CIRI ANTI-ALAHAN *Clinacanthus nutans* (Burm.F) Lindau EKSTRAK DIDEDAH OLEH METABOLOMIK BERASASKAN ^{1}H-NMR

Oleh

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Alahan makanan adalah sejenis reaksi imunologi badan yang tidak normal apabila imun sistem seseorang salah mentafsir makanan sebagai bahan asing yang berbahaya terhadap badan. Walaupun ubat anti-alahan yang sedia ada mampu memberi rawatan yang berkesan, kesan sampingan yang mungkin ke atas manusia telah menarik perhatian para penyelidik untuk mencari alternatif daripada sumber alam semulajadi. Matlamat kajian ini adalah untuk mengenal pasti komposisi metabolit dan menyiasat potensi farmakologi *Clinacanthus nutans,* sejenis herba tradisional dan sayuran tempatan dalam pengurusan alahan makanan dengan menggunakan pendekatan metabolomik berasaskan proton resonans magnetik nuklear (^{1}H-NMR). Bahagian pertama dan kedua kajian ini memberi tumpuan kepada menilai kesan penggunaan bahagian tumbuhan, kaedah pengeringan, sistem pelarut binari (nisbah etanol: air) dan kaedah pengekstrakan yang berbeza terhadap komposisi fitokimia dan bioaktiviti *C. nutans* melalui pendekatan metabolomik. Seterusnya, ekstrak yang optimum akan dinilai untuk kesan ketoksikan akut pada tikus Sprague Dawley pada kepekatan tunggal 5000 mg/kg BW menggunakan kaedah konvensional (pemerhatian tingkah laku visual, pemeriksaan hematologi dan biokimia) dan kaedah terkini (perubahan metabolit dalam serum dan air kencing dengan menggunakan pendekatan metabolomik berasaskan ^{1}H-NMR). Ekstrak tumbuhan yang dipiawaikan selanjutnya dinilai untuk kesan anti-alahan dengan menggunakan tiga kepekatan yang berbeza (125, 500 dan 2000 mg /kg BW) melalui eksperimen *in vivo* anafilaksis yang bersifat sistemik, aktif dan dirangsangi oleh ovalbumin (OVA-ASA). Hasil kajian menunjukkan daun *C. nutans* yang disediakan dengan pengeringan udara, diekstrak dengan air melalui kaedah sonikasi mempunyai jumlah kandungan fenolik (TPC) yang paling tinggi (51.44 ± 0.63 mg GAE/g ekstrak) dan ia juga merupakan ekstrak yang paling berkesan dalam aktiviti perencatan oksida nitrik (NO) (IC_{50}: 190.43 ± 12.26 µg/mL) tanpa memberi kesan sampingan terhadap sel RAW. Clinacoside A dan B,

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clinamide A, B dan C, fitosterol, lupeol, orientin, isoorientin, valine, alanine, asid asetik, dan asid laktik adalah antara sebatian yang berpotensi untuk perencatan NO. Ujian ketoksikan akut menunjukkan tikus yang dirawat berkelakuan normal dan tidak ada morbiditi, kematian atau tanda ketoksikan yang diperhatikan. Oleh itu, LD_{50} untuk ekstrak *C. nutans* adalah melebihi 5000 mg/kg BW. Seterusnya, potensi anti-alahan dari ekstrak *C. nutans* disiasat. Hasil kajian menunjukkan kumpulan tikus yang diberi ekstrak berkepekatan tinggi (2000 mg/kg BW) mempunyai kolerasi positif dengan kumpulan tikus yang normal berbanding dengan kumpulan tikus yang diinduksi oleh ovalbumin. Kajian ini mencadangkan potensi *C. nutans* dalam mengurangkan reaksi alahan adalah disebabkan kebolehannya dalam memodulasi metabolisme karbohidrat, tenaga, lemak, asid amino dan nukleotida tikus yang diinduksi oleh ovalbumin. Kesimpulannya, kajian ini merupakan laporan pertama yang mencadangkan potensi *C. nutans* sebagai agen anti-alahan. Oleh itu, kajian ini mencadangkan satu arah tuju yang baru dalam pembangunan sektor makanan berfungsi atau ubatan yang boleh mengurangkan reaksi alahan dan sebagai asas untuk sebarang kajian yang berkaitan dengan pendekatan metabolomik.

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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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LIST OF ABBREVIATIONS

ABTS	2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid)
ALP	Alkaline phosphatase
ALT	Alanine aminotransferase
ANOVA	Analysis of variance
AST	Aspartate aminotransferase
BW	Body weight
CD_3OD	Deuterated methanol-*d*4
CPMG	Carr–Purcell–Meiboom–Gill
CO_2	Carbon dioxide
CUPRAC	Copper reduction assay
CV-ANOVA	Cross-validation-analysis of variance
d	Day
DAD	Diode array detector
D_2O	Deuterated deuterium oxide
DMSO	Dimethyl sulfoxide
DPPH	2,2-diphenyl-1-picrylhydrazyl
DMEM	Dulbecco's Modified Eagle's Medium
EDTA	Ethylenediaminetetraacetic acid
ESI	Electrospray ionisation
FBS	Fetal bovine serum
FRAP	Ferric reducing antioxidant power
GCMS	Gas chromatography mass spectrometry
GLM	General linear model
h	Hour

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HPLC	High performance liquid chromatography
IC_{50}	Half maximal inhibitory concentration of a substance
IFN-γ	Interferon-gamma
IgE	Immunoglobulin E
IgG	Immunoglobulin G
iNOS	Inducible nitric oxide synthase
i.p.	Intraperitoneal
i.v.	Intravenous
KEGG	Kyoto Encyclopedia of Genes and Genomes
KH_2PO_4	Non-deuterated potassium dihydrogen phosphate
LCMS	Liquid chromatography mass spectrometry
LD_{50}	Lethal dose to kills 50% of a test sample
LDL	Low-density lipoprotein
LPS	Lipopolysaccharide
min	Minute
NaOD	Sodium deuterium oxide
NaN_3	Sodium azide
NMR	Nuclear magnetic resonance
NO	Nitric oxide
NSAIDs	Nonsteroidal Anti-inflammatory Drugs
OECD	Organisation for Economic Cooperation and Development
OPLS-DA	Orthogonal Partial Least Squares–Discriminant Analysis
ORAC	Oxygen radical absorbance capacity
OVA-ASA	Ovalbumin induced active systemic anaphylaxis
PAF	Platelet-activating factor

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PBS	Phosphate buffered saline
PCA	Principal component analysis
PLS	Partial least squares
PLS-DA	Partial least square-discriminate analysis
p.o.	Per os
MS	Mass spectrometry
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
MVA	Multivariate analysis
PC	Principal component
PRESAT	Pre-saturation
TEAC	Trolox equivalent antioxidant capacity
TFC	Total flavonoid content
TLC	Thin layer chromatography
TOSC	Total oxidant scavenging capacity
TPC	Total phenolic content
TRAP	Total radicals trapping antioxidant parameter
TSP	Trimethylsilyl propionic acid-*d*4 sodium salt
UPLC-MS/MS	Ultra-performance liquid chromatography tandem mass spectrometry
uv	Unit variance
VIP	Variable importance in the projection
δ_H	Chemical shift in ppm
^1H	Proton
2D	Two-dimensional

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CHAPTER 1

INTRODUCTION

1.1 Background

According to World Allergy Organization, the rate of occurance of allergic diseases is gradually increased, with an estimation of 0.05-2% lifetime prevalence (Simons et al., 2011). Infants and young Asian children are reported as the most prevalence groups associated with food allergy, with milk and egg among the most commonly identified allergens (Lee et al., 2013). Studies such as Lee et al. (2013) and Pang et al. (2017) stated that egg allergy is predominated over milk allergy in children below the age of 5 in most parts of Asia and western populations (as shown in Figure 1.1 from Lee et al., 2013). Pang et al. (2017) reported a similar finding in a non-randomized analysis of 435 patients (213 children and 222 adults). Children (54%) were more significantly affected by egg allergy compared to adult (13%). In addition, the top three allergens that significantly affecting children were egg white (54%), milk (31%) and soya bean (13%) (Pang et al., 2017).

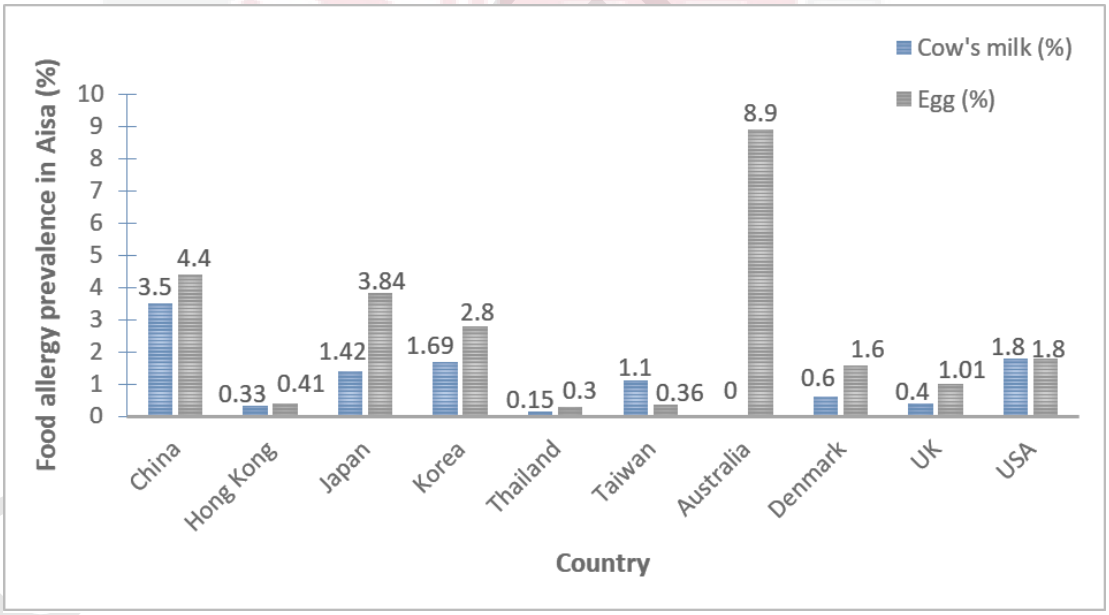

Figure 1.1 : The prevalence of cow's milk and egg allergies in Asia and western countries
(Source: Lee et al., 2013)

In Malaysia, egg is undeniably one of the primary protein sources for Malaysians. Based on the statisitics conducted in year 2015, the egg intake for Malayians was 20.5 kg/year per capita consumption (Government of Malaysia, Department of Statistics Malaysia, 2017). Yadav and Naidu (2015) further revealed that a higher sensitisation

rate to eggs ($p < 0.01$) and cow's milk ($p = 0.044$) was observed in children aged < 2 years when compared to children aged 2–10 years in a food allergen test conducted in Malaysia (Yadav and Naidu, 2015).

Therefore, an effective diagnostic workup of the suspected food allergy and a cautious prophylactic measure in managing the allergy reaction are crucial. Currently, the allergy management relies heavily on food allergen avoidance, emergency preparedness and epinephrine treatment during accidental exposure. Although epinephrine provides effective treatment, the use of synthetic drug elicits adverse effects to the patients especially children (Chalcraft et al., 2014; Dhondalay et al., 2018). Thus, the interest and recognition of natural plants as sources of medicines and alternatives to synthetic drugs have increased worldwide.

Malaysia, a country with rich biodiversity, has been gifted with valuable medicinal plant resources in its tropical rainforest. *Clinacanthus nutans* (Burm. f) Lindau, a small perennial shrub that is native to Southeast Asia has been chosen for this project to study its potential use in mitigating the allergic reactions. It is classified under the family of Acanthaceae, and it is commonly used as a traditional herbal medicine and a food source. Since 1987, *C. nutans* has been promoted as a valuable medicinal herb to be developed by the government of Thailand and Malaysia (Panyakom, 2006; Putwatana et al., 2009).

To date, triterpenoids, *C*-glycosyl flavones, sulfur-containing glucosides, sulfur-containing compounds, monoacylmonogalactosylglycerol, a mixture of nine cerebrosides and chlorophyll derivatives are the main phytochemicals identified in *C. nutans* (Alam et al., 2016a; Sakdarat et al., 2009; Teshima et al., 1997, 1998; Tu et al., 2014; Tuntiwachwuttikul et al., 2004). *C. nutans* has a long history of use for treating snake bites, herpes infection, cancer, burns and scalds, dysentery, diabetes and skin disorders in Thailand, Indonesia, Singapore and Malaysia (Alam et al., 2016a; Ho et al., 2013; Levey, 1969; Siew et al., 2014; Thawaranantha et al., 1992). Nevertheless, data reported in the literature shows that there is still a vast gap to fill between the traditional uses and the scientifically verified study of *C. nutans* as an antidote for treating allergy reaction. In addition, there is also deficient of study that extensively profiles the phytochemical composition of *C. nutans* and correlates to its anti-allergy potency.

Meanwhile, recent technological advances have brought the possibility of moving traditional biological analytical techniques toward more sophisticated and high-throughput approaches (Dhondalay et al., 2018). First put forward by Nicholson et al. (1999), metabolomics, one of the high throughput omics-techniques, offers a great alternative (Chen et al., 2016). Unlike other -omics approaches such as proteomics and genomics, metabolomics provides a snapshot that reflects the overall dynamic metabolism status of an organism at the time of sampling (Wang et al., 2017). The ability to simultaneously profile, quantify and integrate a large number of low-molecular metabolites in a single experiment further highlights the attractive

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advantage of metabolomics (Wang et al., 2017). Metabolomics, with its holistic approach is thus in agreement with natural herbal medicine. It is also found suitable to study food-related anaphylaxis as it is capable of detecting changes that occur within minutes (the time-frame of anaphylaxis) (Liu et al., 2014).

In order to fully exploit the pharmacological effect of the herbal extract in food allergy study, animal model is essential, especially when systemic allergic response involves the whole body. Murine model has emerged as an appropriate approach over the use of human subjects in food allergy studies as it allows the induction of specific allergens under controlled conditions and genetic backgrounds, which is not possible in human studies (Oyoshi et al., 2014).

Feasible and efficient authentication and toxicity testing are also important in evaluating the identity and safety profile of a plant material before it is deemed safe for human consumption (Arome and Chinedu, 2014). To date, few toxicity profiles of *C. nutans* have been established. Nevertheless, it is valuable to delineate the toxicity concerns associated with *C. nutans* extract based on endogenous biological metabolite variations in rats after extract administration.

In addition, the pharmacological quality and efficacy of plants or plant organs are largely dependent on the distribution of the phytochemical composition in the plant matrix. Plant post-harvest processing methods, such as the drying method, the nature of extracting solvent and extraction method, are important factors that could affect the metabolites composition in a plant even within the same species due to different thermal sensitivities and polar affinities of the metabolites. There is no single and standard plant post-harvest processing method that can simultaneously elucidate all types of metabolites from natural products; each method presents advantages and drawbacks. Hence, the effect of plant processing methods on the plant material should be evaluated and characterised.

Therefore, the present study aimed for the first time to evaluate the mechanism of action of *C. nutans* in alleviating food allergy reaction using proton nuclear magnetic resonance (^{1}H-NMR) metabolomics approach. This study was also the first attempt that utilised ^{1}H-NMR metabolomics approach in assessing the acute toxicity effect of *C. nutans*. Prior to the study of anti-allergy properties of the plant, the effect of plant post-harvest processing parameters including the dryings, extraction solvents and extraction methods, and the choice of plant part on the phytochemicals characterisation and bioactivity of *C. nutans* were carefully analysed.

1.2 Problem statements

Increasing prevalence of food allergy is always associated with the problem of limited diagnosis and treatment methods. At the same time, there is also an inherent jeopardy arising from the over reliance on the synthetic medicines in curing food allergy. This

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rises the interest of science community in identifying the optimal natural herbal plants as the alternative therapeutic sources. Malaysia, with a vast number of herbal plants present in the tropical forest, offers a unique opportunity to study plants with potential medicinal value, particularly as an allergy antidote. *C. nutans*, a well-known local herb and vegetable, is commonly used to treat skin rashes. However, to date, there is no studies that characterise and verify its anti-allergy effect using systematic scientific approaches. Limited studies have illustrated the relationship between the phytochemical composition of *C. nutans* and its anti-allergy properties. Meanwhile, previous toxicity studies of *C. nutans* extract were limited to conventional behavioural observation and biochemical evaluation. None of the research works has discussed the safety profile of *C. nutans* through detailed illustration of the endogenous metabolites status of the extract-treated organisms. In fact, little is known on how the choice of plant part and plant post-harvest processing parameters affects the metabolite composition, and consequently the bioactivities of *C. nutans*.

1.3 Objectives

The present study was designed to gain a better understanding of the mechanism of action, role and potency of *C. nutans* in treating food allergic disorder. To achieve these goals, several specific objectives were proposed and ^{1}H-NMR based metabolomics was utilised as the main analytical approach in all the objectives.

The five specific objectives are listed below:

1. To determine the effect of different plant parts, dryings and extraction methods on the phytochemical composition and bioactivity of *C. nutans* using ^{1}H-NMR-based metabolomics.
2. To evaluate the effect of different solvents and extraction methods on the phytochemical composition and nitric oxide inhibitory activity of *C. nutans.*
3. To identify and quantify the phytochemicals using ^{1}H-NMR-based metabolomics and HPLC–MS/MS analysis.
4. To evaluate the acute toxicity profile of 5000 mg/kg air dried-sonicated *C. nutans* water leaf extract using hematological and biochemical analyses and ^{1}H-NMR-based metabolomics approach.
5. To identify the biomarkers and elucidate the mechanism of action of *C. nutans* water leaf extract in an *in vivo* ovalbumin-induced active systemic anaphylaxis model.

REFERENCES

Abdul-Hamid, N.A., Mediani, A., Maulidiani, M., Abas, F., Ismail, I.S., Shaari, K., & Lajis, N.H. (2016). Discrimination and nitric oxide inhibitory activity correlation of Ajwa dates from different grades and origin. *Molecules, 21*(11), 1-14.

Abdul Rahim, M.H., Zakaria, Z.A., Mohd Sani, M.H., Omar, M.H., Yakob, Y., Cheema, M.S., Ching, S.M., Ahmad, Z., & Abdul Kadir, A. (2016). Methanolic extract of *Clinacanthus nutans* exerts antinociceptive activity via the opioid/nitric oxide-mediated, but cGMP-independent pathways. *Evidence-based Complementary and Alternative Medicine,* 2016, 1–11.

Abdullah, S., Shaari, A.R., & Azimi, A. (2012). Effect of drying methods on metabolites composition of misai kucing (*Orthosiphon stamineus*) leaves. *APCBEE Procedia, 2*, 178–182.

Abu Bakar Sajak, A., Mediani, A., Maulidiani, Mohd Dom, N.S., Machap, C., Hamid, M., Ismail, A., Khatib, A., & Abas, F. (2017). Effect of *Ipomoea aquatica* ethanolic extract in streptozotocin (STZ) induced diabetic rats via ^{1}H NMR-based metabolomics approach. *Phytomedicine, 36*, 201–209.

Agustina, S. (2011). *Isolation of flavonoids as antioxidant from Dandang Gendis (Clinacanthus nutans) leaves* (Unpublished bachelor degree thesis). Bogor Agricultural University, Bogor, Indonesia.

Ainsworth, E.A., & Gillespie, K.M. (2007). Estimation of total phenolic content and other oxidation substrates in plant tissues using Folin–Ciocalteu reagent. *Nature Protocols, 2*(4), 875–877.

Aktan, F. (2004). *i*NOS-mediated nitric oxide production and its regulation. *Life Sciences, 75*(6), 639–653.

Alam, A., Ferdosh, S., Ghafoor, K., Hakim, A., Juraimi, A.S., Khatib, A., & Sarker, Z.I. (2016a). *Clinacanthus nutans*: A review of the medicinal uses, pharmacology and phytochemistry. *Asian Pacific Journal of Tropical Medicine, 9*(4), 402–409.

Alam, A., Zaidul, I.S.M., Ghafoor, K., Sahena, F., Ali, E., Kamaruzzaman, B.Y., Jahurul, M.H.A., Khatib, A., Alam, A., Zaidul, I.S.M., Ghafoor, K., Sahena, F., & Ali, E. (2016b). Identification of bioactive compounds with GC-Q-TOF-MS in the extracts from *Clinacanthus nutans* using subcritical carbon dioxide extraction. *Separation Science and Technology, 52*(5), 852–863.

© COPYRIGHT UPM

Amiraslani, B., Sabouni, F., Abbasi, S., Nazem, H., & Sabet, M. (2012). Recognition of betaine as an inhibitor of lipopolysaccharide-induced nitric oxide production in activated microglial cells. *Iranian Biomedical Journal, 16*(2), 84–89.

Anastasiadi, M., Zira, A., Magiatis, P., Haroutounian, S.A., Skaltsounis, A.L., & Mikros, E. (2009). ^{1}H NMR-based metabonomics for the classification of Greek wines according to variety, region, and vintage. comparison with HPLC data. *Journal of Agricultural and Food Chemistry, 57*(23), 11067–11074.

Andhare, R.N., Raut, M.K., & Naik, S.R. (2012). Evaluation of antiallergic and anti-anaphylactic activity of ethanolic extract of *Sanseveiria trifasciata* leaves (EEST) in rodents. *Brazilian Journal of Pharmacognosy, 142*, 627–633.

Andrade Júnior, C. M. (2018). Metabolism during fasting and starvation: Understanding the basics to glimpse new boundaries. *Journal of Nutrition and Dietetics*, *1*(1), 1000e102.

Anilkumar, K., Reddy, G. V., Azad, R., Yarla, N.S., Dharmapuri, G., Srivastava, A., Kamal, M.A., & Pallu, R. (2017). Evaluation of anti-inflammatory properties of isoorientin isolated from tubers of *Pueraria tuberosa*. *Oxidative Medicine and Cellular Longevity, 2017*, 1–10.

Antwi, A.O., Obiri, D.D., & Osafo, N. (2017). Stigmasterol modulates allergic airway inflammation in guinea pig model of ovalbumin-induced asthma. *Mediators of Inflammation, 2017*, 1-11.

Anwar, F, & Przybylski, R. (2012). Effect of solvents extraction on total phenolics and antioxidant activity of extracts from flaxseed (*Linum usitatissimum* L.). *Acta Scientiarum Polonorum Technologia Alimentaria*, *11*(3), 293–301.

Arome, D., & Chinedu, E. (2014). The importance of toxicity testing. *Journal of Pharmaceutical and BioScienc*e, *4*(2013), 146–148.

Arullappan, S., Rajamanickam, P., Thevar, N., & Kodimani, C. (2014). *In vitro* screening of cytotoxic, antimicrobial and antioxidant activities of *Clinacanthus nutans* (Acanthaceae) leaf extracts. *Tropical Journal of Pharmaceutical Research, 13*(9), 1455.

Atta-ur-Rahman, Zareen, S., Choudhary, M.I., Akhtar, M, N., & Ngounou, F.N. (2008). A triterpenoidal saponin and sphingolipids from *Pteleopsis hylodendron*. *Phytochemistry*, *69*, 2400–2405.

Azizan, K.A., Baharum, S.N., Ressom, H.W., Noor, N.M., & States, U. (2012). GC-MS analysis and PLS-DA validation of the trimethyl silyl-derivatization techniques. *American Journal of Applied Sciences, 9*(7), 1124–1136.

© COPYRIGHT UPM

Baeza, M.L., & Zubeldia, J.M. (2007). Immunology of anaphylaxis: Lessons from murine models. *Current Allergy and Asthma Reports, 7*, 49–55.

Barth, D., & Wiebe, M.G. (2017). Enhancing fungal production of galactaric acid. *Applied Microbiology and Biotechnology, 101*(10), 4033–4040.

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

Beg, S., Hasan, H., Hussain, M.S., Swain, S., & Barkat, Ma. (2011). Systematic review of herbals as potential anti-inflammatory agents: Recent advances, current clinical status and future perspectives. *Pharmacognosy Reviews, 5*(10), 120.

Bellik, Y., Boukraâ, L., Alzahrani, H.A., Bakhotmah, B.A., Abdellah, F., Hammoudi, S.M., & Iguer-Ouada, M. (2013). Molecular mechanism underlying anti-inflammatory and anti-allergic activities of phytochemicals: An update. *Molecules, 18*(1), 322–353.

Bellik, Y., M. Hammoudi, S., Abdellah, F., Iguer-Ouada, M., & Boukraa, L. (2012). Phytochemicals to prevent inflammation and allergy. *Recent Patents on Inflammation & Allergy Drug Discovery, 6*(2), 147–158.

Ben-Shoshan, M., & Clarke, A.E. (2011). Anaphylaxis: Past, present and future. *Allergy: European Journal of Allergy and Clinical Immunology*, *66*, 1–14.

Berkes, E.A. (2003). Anaphylactic and anaphylactoid reactions to aspirin and other NSAIDs. *Clinical Reviews in Allergy and Immunology, 24*, 137–147.

Bernhoft, A. (2010). Bioactive compounds in plants – benefits and risks for man and animals, in: *The Norwegian Academy of Science and Letters Bioactive.* Oslo, pp. 18–29.

Boongerd, K. (1967). *The chemical constituents of Clinacanthus burmanii* (Unpublished master science thesis). Chulalongkorn University, Bangkok, Thailand.

Brand-Williams, W., Cuvelier, M.E., & Berset, C. (1995). Use of a free radical method to evaluate antioxidant activity. *LWT–Food Science Technology, 28*(1), 25–30.

Brito, F.A., Lima, L.A., Ramos, M.F.S., Nakamura, M.J., Cavalher-Machado, S.C., Siani, A.C., Henriques, M.G.M.O., & Sampaio, A.L.F. (2007). Pharmacological study of anti-allergic activity of *Syzygium cumini* (L.) Skeels. *Brazilian Journal of Medical and Biological Research, 40*(1), 105–115.

Brzóska, M.M., Moniuszko-Jakoniuk, J., Piłat-Marcinkiewicz, B., & Sawicki, B. (2003). Liver and kidney function and histology in rats exposed to cadmium and ethanol. *Alcohol and Alcoholism, 38*(1), 2–10.

© COPYRIGHT UPM

Caubet, J.C., & Wang, J. (2011). Current understanding of egg allergy. *Pediatric Clinics of North America, 58*(2), 427–443.

Cauwels, A., Janssen, B., Buys, E., Sips, P., & Brouckaert, P. (2016). Anaphylactic shock depends on PI3K and eNOS-derived NO. *Journal of Clinical Investigation, 116*(8), 2244–2251.

Chadwick, D.J., & Julie, W. (2008). Secondary metabolites: Their function and evolution.

Chakravortty, D., Koide, N., Kato, Y., Sugiyama, T., Mu, M.M., Yoshida, T., & Yokochi, T. (2000). The inhibitory action of butyrate on lipopolysaccharide-induced nitric oxide production in RAW 264.7 murine macrophage cells. *Journal of Endotoxin Research, 6(3)*, 243–247.

Chalcraft, K.R., Kong, J., Waserman, S., Jordana, M., & Mccarry, B.E. (2014). Comprehensive metabolomic analysis of peanut-induced anaphylaxis in a murine model. *Metabolomics, 10*, 452–460.

Chamratpan, S., & Homchuen, S.A. (2005). Ethnobotany in upper northeastern Thailand. *Acta Horticulturae, 675*, 67–74.

Charerntantanakul, W., & Kawaree, R. (2010). Effects of medicinal plants extracts on interleukin-10 and tumor necrosis factor alpha gene expressions in porcine peripheral blood mononuclear cells. *Chiang Mai Veterinary Journal, 8*(2), 93–103.

Charuwichitratana, S., Wongrattanapass, N., Timpatanapong, P., & Bunjob, M. (1996). *Herpes zoster*: Treatment with *Clinacanthus nutans* cream. *International Journal of Dermatology, 35*(9), 665–666.

Chatham, J. C. (2002). Lactate - The forgotten fuel! *Journal of Physiology*, *542*(2), 333.

Chavalittumrong, P., Attawish, A., Rugsamon, P., & Chuntapet, P. (1995). Toxicological study of *Clinacanthus nutans* (Burm.f.) Lindau. *Bulletin of the Department of Medical Sciences*, *37*(4), 323-338.

Cheeptham, N., & Towers, G.H.N. (2002). Light-mediated activities of some Thai medicinal plant teas. *Fitoterapia, 73*, 651–662.

Chelyn, J.L., Omar, M.H., Mohd Yousof, N.S.A., Ranggasamy, R., Wasiman, M.I., & Ismail, Z. (2014). Analysis of flavone *C*-glycosides in the leaves of *Clinacanthus nutans* (Burm. F.) Lindau by HPTLC and HPLC-UV/DAD. *The Scientific World Journal, 2014*, 1–6.

© COPYRIGHT UPM

Chemat, F., Huma, Z., & Khan, M.K. (2011). Applications of ultrasound in food technology : Processing, preservation and extraction. *Ultrasonics Sonochemistry, 18*, 813–835.

Chen, D.Q., Chen, H., Chen, L., Tang, D.D., Miao, H., & Zhao, Y.Y. (2016). Metabolomic application in toxicity evaluation and toxicological biomarker identification of natural product. *Chemico-Biological Interactions, 252*, 114–130.

Chen, H., Song, Q., Diao, X., & Zhou, H. (2016). Proteomic and metabolomic analysis on the toxicological effects of Benzo[a]pyrene in pearl oyster *Pinctada martensii*. *Aquatic Toxicology, 175*, 81–89.

Chen, H. J., Inbaraj, B. S., & Chen, B. H. (2012). Determination of phenolic acids and flavonoids in *Taraxacum formosanum* Kitam by liquid chromatography-tandem mass spectrometry coupled with a post-column derivatization technique. *International Journal of Molecular Sciences*, *13*(1), 260-285.

Cheong, B.E., Ho, S.Y., Dickens Wong, V.F., Wilson Yong, T.L., Teoh, P.L., & How, S.E. (2013). Chemical profiling of Sabah snake grass, *Clinacanthus nutans*, in: Proceedings of the 11th Seminar on Science & Technology.

Cherdchu, C., Poopyruchpong, N., Adchariyasucha, R., & Ratababanangkoon, K. (1977). The absence of antagonism between extracts of *Clinacanthus nutans* Burm. and *Naja naja siamensis* venom. *Southeast Asian Journal of Tropical Medicine and Public Health, 8* (2), 249–254.

Chewprecha, W., Srisodsuk, M., & Kasetsart, U. (2006). *Cytotoxicity of Thai medicinal plant extracts on mammalian cells*. Paper presented at the Proceedings of 44th Kasetsart University Annual Conference: Agricultural Science: Carrying Forward the Royal Bio-Energy Initiative, Kasetsart University, Bangkok, Thailand.

Chin, J.H., Akowuah, G.A., Sabu, M.C., & Khalivulla, S.I. (2014). Sub-acute (28 days) toxicity study of methanol leaves extract of *Clinacanthus nutans* in rats. *International Journal of Pharmacy, 4*(2), 61–69.

Chitarrini, G., Soini, E., Riccadonna, S., Franceschi, P., Zulini, L., Masuero, D., Vecchione, A., Stefanini, M., Di Gaspero, G., Mattivi, F., & Vrhovsek, U. (2017). Identification of biomarkers for defense response to *Plasmopara viticola* in a resistant grape variety. *Frontiers in Plant Science, 8*, 1–11.

Chomnawang, M.T., Surassmo, S., Nukoolkarn, V.S., & Gritsanapan, W. (2005). Antimicrobial effects of Thai medicinal plants against acne-inducing bacteria. *Journal of Ethnopharmacology, 101*, 330–333.

© COPYRIGHT UPM

Chomnawang, M.T., Surassmo, S., Nukoolkarn, V.S., & Gritsanapan, W. (2007). Effect of *Garcinia mangostana* on inflammation caused by *Propionibacterium acnes*. *Fitoterapia, 78*(6), 401–408.

Chomnawang, M.T., Surassmo, S., Wongsariya, K., & Bunyapraphatsara, N. (2009a). Antibacterial activity of Thai medicinal plants against methicillin-resistant *Staphylococcus aureus*. *Fitoterapia, 80*(2), 102–104.

Chomnawang, M.T., Trinapakul, C., & Gritsanapan, W. (2009b). *In vitro* antigonococcal activity of *Coscinium fenestratum* stem extract. *Journal of Ethnopharmacology 122*(3), 445–449.

Chonlasin, K., Visetson, S., Phanwichien, K., & Foongladda, S. (2002). *Ability of Clinacanthus nutans (Burm.F.) Lindau of inhibition Newcastle disease virus (NDV) infection in cell culture.* Paper presented at the Proceedings of 41th Kasetsart University Annual Conference: Animals, Veterinary Medicine, Kasetsart University, Bangkok, Thailand

Choy, W.L.V., Hwi, K.K., & Dublin, N. (2011). Effects of *Clinacanthus nutans* and mebeverine hydrochloride on bladder activity. *Journal of Men's Health, 8*(S1), S119-S120.

Contini, M., Baccelloni, S., Massantini, R., & Anelli, G. (2008). Extraction of natural antioxidants from hazelnut (*Corylus avellana* L.) shell and skin wastes by long maceration at room temperature. *Food Chemistry, 110*, 659–669.

Cory, J.G., & Cory, A.H. (2006). Critical roles of glutamine as nitrogen donors in purine and pyrimidine nucleotide synthesis: Asparaginase treatment in childhood acute lymphoblastic leukemia. *In vivo, 20*, 587–590.

Cos, P., Vlietinck, A. J., Berghe, D. Vanden, & Maes, L. (2006). Anti-infective potential of natural products: How to develop a stronger *in vitro* "proof-of-concept." *Journal of Ethnopharmacology*, *106*(3), 290–302.

Dampawan, P. (1976). Studies of the chemical constituents of *Clinacanthus nutans* (acanthaceae) and *Zingiber cassumunar* Roxb. (zingiberaceae). Mahidol University.

Dampawan, P., Huntrakul, C., & Reutrakul, V. (1977). Constituents of *Clinacanthus nutans* and the crystal structure of lup-20(29)-ene-3-one. *Journal of the Science Society of Thailand, 3*(1), 14–26.

Dan, P.N.M. (2014). *Evaluation of biological activities of Clinacanthus nutans (Burm. F.) Lindau* (Unpublished bachelor science thesis). Vietnam National University, Hanoi, Vietnam.

© COPYRIGHT UPM

de Oliveira, D.M., Siqueira, E.P., Nunes, Y.R.F., & Cota, B.B. (2013). Flavonoids from leaves of *Mauritia flexuosa*. *Brazilian Journal of Pharmacognosy, 23*(4), 614–620.

Denning, D., & Webster, A. (1987). Detrimental effect of propylene glycol on natural killer cell and neutrophil function. *Journal of Pharmacy and Pharmacology, 39*, 236–238.

Dhondalay, G.K., Rael, E., Acharya, S., Zhang, W., Sampath, V., Galli, S.J., Tibshirani, R., Boyd, S.D., Maecker, H., Nadeau, K.C., & Andorf, S. (2018). Food allergy and omics. *Journal of Allergy and Clinical Immunology, 141*(1), 20–29.

Direkbusarakom, S., Ezura, Y., Yoshimizu, M., & Herunsalee, A. (1998a). Efficacy of Thai traditional herb extracts against fish and shrimp pathogenic bacteria. *Fish Pathology, 33*(4), 437–441.

Direkbusarakom, S., Herunsalee, A., Yoshimizu, M., & Ezura, Y. (1996). Antiviral activity of several Thai traditional herb extracts against fish pathogenic viruses. *Fish Pathology, 31*(4), 209–213.

Direkbusarakom, S., Ruangpan, L., Ezura, Y., & Yoshimizu, M. (1998b). Protective efficacy of *Clinacanthus nutans* on yellow-head disease in black tiger shrimp (*Penaeus monodon*). *Fish Pathology, 33*(4), 401–404.

Dollah, M.A., Parhizkar, S., Abdul Latiff, L., & Hassan, M.H. (2013). Toxicity effect of *Nigella sativa* on the liver function of rats. *Advanced Pharmaceutical Bulletin, 3*(1), 97–102.

Dzhanashvili, G. (1967). Hygienic substantiation of the maximum permissible content of dimethylamine in water bodies. *Gigiena I Sanitariia, 6*, 16.

Eriksson, L., Byrne, T., Johansson, E., Trygg, J., & Vikström, C. (2013). *Multi-and megavariate data analysis basic principles and applications* (Third). Umetrics Academy, Sweden.

Eriksson, L., Johansson, E., Kettaneh-Wold, N., Trygg, J., Wikstrom, C., & Wold, S. (2006). *Multi- and megavariate data analysis part I: Basic principles and applications*. Umetrics, Inc, Umea, Sweden.

Escribese, M.M., Rosace, D., Chivato, T., Tahia D. Fernández, Angel L. Corbí, & Barber, D. (2017). Alternative anaphylactic routes : The potential role of macrophages. *Frontiers in Immunology, 8*, 1–7.

Escudero, N., Marhuenda-Egea, F.C., Ibanco-Canẽte, R., Zavala-Gonzalez, E.A., & Lopez-Llorca, L.V. (2015). A metabolomic approach to study the rhizodeposition in the tritrophic interaction: Tomato, *Pochonia chlamydosporia* and *Meloidogyne javanica*. *Metabolomics, 10*(5), 788–804.

© COPYRIGHT UPM

Esmailli, K., Alsuede, F., Aisha, A., Shafaei, A., & Ismail, Z. (2013, March). *Preliminary phytochemical analysis and cytotoxicity studies of Clinacanthus nutans (Sabah snake grass)*. Paper presented at the Proceedings of the International Conference on Natural Products, Shah Alam Conventional Center, Malaysia.

Evans, H., Killoran, K.E., & Mitre, E. (2014). Measuring local anaphylaxis in mice. *Journal of Visualized Experiments*, 1–6.

Evora, P.R.B., & Simon, M.R. (2007). Role of nitric oxide production in anaphylaxis and its relevance for the treatment of anaphylactic hypotension with methylene blue. *Annals of Allergy, Asthma and Immunology, 99*(4), 306–313.

Farsi, E., Abdul Majid, A.S., & Abdul Majid, A.M.S. (2016a). *Clinacanthus nutans*, yesterday's practice, and future's drug: A comprehensive review. *American Journal of Phytomedicine and Clinical Therapeutics, 4*(4), 113–126.

Farsi, E., Esmailli, K., Shafaei, A., Moradi Khaniabadi, P., Al Hindi, B., Khadeer Ahamed, M.B., Sandai, D., Abdul Sattar, M., Ismail, Z., Abdul Majid, A.M.S., & Abdul Majid, A.S. (2016b). Mutagenicity and preclinical safety assessment of the aqueous extract of *Clinacanthus nutans* leaves. *Drug and Chemical Toxicology, 545*, 1–13.

Fathi, E., & Sefidkon, F. (2012). Influence of drying and extraction methods on yield and chemical composition of the essential oil of *Eucalyptus sargentii*. *Journal of Agricultural Science and Technology, 14*(5), 1035–1042.

Fernando, I.P.S., Nah, J.W., & Jeon, Y.J. (2016). Potential anti-inflammatory natural products from marine algae. *Environmental Toxicology and Pharmacology, 48*, 22–30.

Finkelman, F.D. (2007). Anaphylaxis: Lessons from mouse models. *Journal of Allergy and Clinical Immunology, 120*(3), 506–515.

Folin, O., & Ciocalteau, V. (1927). Tyrosine and tryptophane determination in proteins. *Journal of Biological Chemistry, 73*(2), 627–648.

Folin, O., & Denis, W. (1912). Phosphotungstic-phosphomolybdic compounds as color reagents. *The Journal of Biological Chemistry, 12*, 239–243.

Fong, S.Y. (2015). *Genetic, phytochemical and bioactivity studies of Clinacanthus nutans (Burm. F.) Lindau (Acanthaceae)* (Unpublished doctoral dissertation). RMIT University, Malaysia.

Fong, S.Y., Piva, T., Dekiwadia, C., Urban, S., & Huynh, T. (2016). Comparison of cytotoxicity between extracts of *Clinacanthus nutans* (Burm. f.) Lindau leaves from different locations and the induction of apoptosis by the crude methanol leaf extract in D24 human melanoma cells. *BMC Complementary and Alternative Medicine, 16*, 368.

© COPYRIGHT UPM

Fong, S.Y., Piva, T., Urban, S., & Huynh, T. (2014). Genetic homogeneity of vegetatively propagated *Clinacanthus nutans* (Acanthaceae). *Journal of Medicinal Plant Research, 8*(25), 903–914.

Forstermann, U., & Sessa, W.C. (2012). Nitric oxide synthases : Regulation and function. *European Heart Journal, 33*, 829–837.

Fredholm, B.B., & Strandberg, K. (1975). Some metabolic consequences of the anaphylactic reaction in the rabbit. *Acta Physiologica, 93*(1), 52–58.

Galli, S.J., Tsai, M., & Piliponsky, A.M. (2008). The development of allergic inflammation. *Nature, 454*(7203), 445–454.

George, M., & Joseph, L. (2010). Anti-allergic, anti-pruritic, and anti-inflammatory activities of *Centella asiatica* extracts. *African Journal of Traditional, Complementary and Alternative Medicines, 6*(4), 554–559.

Ghasemzadeh, A., Nasiri, A., Jaafar, H.Z.E., Baghdadi, A., & Ahmad, I. (2014). Changes in phytochemical synthesis, chalcone synthase activity and pharmaceutical qualities of sabah snake grass (*Clinacanthus nutans* L.) in relation to plant age. *Molecules, 19*(11), 17632–17648.

Gloyn, A.L. (2004). Betaine in human nutrition. *The American Journal of Clinical Nutrition, 80*, 539–49.

Goonasakaran, S. (2013). *Preliminary antimicrobial and phytochemical analysis of Clinacanthus nutans and Azadirachta indica* (Unpublished master degree). Universiti Teknologi Malaysia, Johor, Malaysia.

Government of Malaysia, Department of Statistics Malaysia. (2017). Supply and utilization accounts selected agricultural commodities, Malaysia 2012-2016. Retrieved from
https://www.dosm.gov.my/v1/index.php?r=column/cthemeByCat&cat=164&bul_id=UDROQllWME5ETGZrcUE5VnAzcHJEQT09&menu_id=Z0VTZGU1UHBUT1VJMFlpaXRRR0xpdz09

Grabacka, M., Pierzchalska, M., Dean, M., & Reiss, K. (2016). Regulation of ketone body metabolism and the role of PPARα. *International Journal of Molecular Sciences, 17*(2093), 1–24.

Grasso, D., Chin, Y.-P., JR, & W.J.W. (1990). Structural and behavioral characteristics of a commercial humic acid and natural dissolved aquatic organic matter. *Chemosphere, 21*, 1181–1197.

Green, L., Wagner, D., Glogowski, J., Skipper, P., Wishnok, J., & Tannenbaum, S. (1982). Analysis of nitrate, nitrite and (^{15}N) nitrate in biological fluids. *Analytical Biochemistry, 126*(1), 131–138.

© COPYRIGHT UPM

Guo, Y., Hedqvist, P., & Gustafsson, L.E. (2001). Absence of mast cell involvement in active systemic anaphylaxis in rats. *European Journal of Pharmacology, 430*(2-3), 305–310.

Gutierrez, E. N., & Lamberti, V. (1978). *U.S. Patent No. 4,123,458*. Washington, DC: U.S. Patent and Trademark Office.

Hajimehdipoor, H., Adib, N., Khanavi, M., Mobli, M., Amin, G.R., & Moghadam, H. (2012). Comparative study on the effect of different methods of drying on phenolics content and antioxidant activity of some edible plants. *International Journal of Pharmaceutical Sciences and Research, 3*(10), 3712–3716.

Hamid, H.A., & Yahaya, I.H. (2016). Cytotoxicity of *Clinacanthus nutans* extracts on human hepatoma (HepG2) cell line. *International Journal of Pharmacy and Pharmaceutical Sciences, 8*(10), 8–10.

Hamid, H.A., Yahya, I.H., Yusoff, M.M., & Zareen, S. (2016). Bioassay-guided isolation and antioxidant activity of sulfur-containing compounds from *Clinacanthus nutans*. *Journal of the Chinese Chemical Society, 63*(12), 1033–1037.

Handa, S.S., Khanuja, S.P.S., Longo, G., & Rakesh, D.D. (2008). Extraction techniques of medicinal plants. *International Centre for Science and High Technology*.

Haroun, M.F., & Al-Kayali, R.S. (2016). Synergistic effect of *Thymbra spicata* L. extracts with antibiotics against multidrug- resistant *Staphylococcus aureus* and *Klebsiella pneumoniae* strains. *Iranian Journal of Basic Medical Sciences, 19*(11), 1193–1200.

Havelund, J.F., Heegaard, N.H.H., Færgeman, N.J.K., & Gramsbergen, J.B. (2017). Biomarker research in parkinson's disease using metabolite profiling. *Metabolites, 7*(42), 1-18.

He, L., Xu, Z., Yao, K., Wu, G., Yin, Y., Nyachoti, C.M., & Kim, S.W. (2015). The physiological basis and nutritional function of alpha-ketoglutarate. *Current Protein & Peptide Science, 16*(7).

Hierholzer, B.C., Harbrecht, B., Menezes, J.M., Kane, J., Macmicking, J., Nathan, C.F., Peitzman, A.B., Billiar, T.R., & Tweardy, D.J. (1998). Essential role of induced nitric oxide in the initiation of the inflammatory response after hemorrhagic shock. *Journal of Experimental Medicine, 187*(6), 917–928.

Ho, S.Y., Tiew, W.P., Madhavan, P., Abdul Shukkoor, M.S., & Akowuah, G.A. (2013). Phytochemical analysis and antibacterial activity of methanolic extract of *Clinacanthus nutans* leaf. *International Journal of Drug Development and Research, 5*(3), 349–355.

© COPYRIGHT UPM

Hong-fang, J., Ai-lin, D., Ling-wen, Z., Chun-yang, X., Ming-duo, Y., & Fang-fang, L. (2012). Effects of drying methods on antioxidant properties in *Robinia pseudoacacia* L. flowers. *Journal of Medicinal Plants Research*, *6*, 3233–3239.

Horgan, R.P., & Kenny, L.C. (2011). SAC review " Omic " technologies : proteomics and metabolomics. *The Obstetrician & Gynaecologist, 13*, 189–195.

Hossain, M., Barry-ryan, C., & Martin-diana, A. B. (2010). Effect of drying method on the antioxidant capacity of six Larniaceae herbs. *Food Chemistry*, *123*(1), 85–91.

Hu, X., Wu, G., Zhang, M., Pan, S.Q., Wang, R.R., Ouyang, J.H., Liu, J.G., Chen, Z.Y., Tian, H.L., & Liu, D.B. (2012). GC-MS-based metabolic profiling reveals metabolic changes in anaphylaxis animal models. *Analytical and Bioanalytical Chemistry, 404,* 887–893.

Huang, D., Guo, W., Gao, J., Chen, J., & Olatunji, J.O. (2015). *Clinacanthus nutans* (Burm. F.) Lindau ethanol extract inhibits hepatoma in mice through upregulation of the immune response. *Molecules, 20*(9), 17405–17428.

Huang, D., Li, Y., Cui, F., Chen, J., Sun, J. (2016). Purification and characterization of a novel polysaccharide-peptide complex from *Clinacanthus nutans* Lindau leaves. *Carbohydrate Polymers, 137*, 701–708.

Huo, Y., Kamal, G.M., Wang, J., Liu, H., Zhang, G., Hu, Z., Anwar, F., & Du, H. (2017). ^{1}H NMR-based metabolomics for discrimination of rice from different geographical origins of China. *Journal of Cereal Science, 76*, 243–252.

Hussain, M.S., Azam, F., Ahamed, K.F.H.N., Ravichandiran, V., & Alkskas, I. (2016). Anti-endotoxin effects of terpenoids fraction from *Hygrophila auriculata* in lipopolysaccharide-induced septic shock in rats. *Pharmaceutical Biology, 54*(4), 628–636.

Hussein, R.R.S., Soliman, R.H., Abdelhaleem Ali, A.M., Tawfeik, M.H., & Abdelrahim, M.E.A. (2013). Effect of antiepileptic drugs on liver enzymes. *Beni-Suef University Journal of Basic and Applied Sciences, 2*(1), 14–19.

Illingworth, J. (2012). Specialised metabolism of differentiated tissues. Retrieved from http://www.bmb.leeds.ac.uk/teaching/icu3/lecture/22/index.htm

Jamal, P., Barkat, A.A., & Amid, A. (2010). Distribution of phenolics in various Malaysian medicinal plants. *Journal of Applied Sciences, 10*(21), 2658–2662.

Janeway, C.A., Travers, P., Walport, M., & Shlomchik, M.J. (2001). *The immune system in health and disease* (Fifth). Garland Science, New York.

Jang, H.H., Cho, S.Y., Kim, M.J., Kim, J.B., Lee, S.H., Lee, M.Y., & Lee, Y.M. (2016). Anti-inflammatory effects of *Salvia plebeia* R . Br extract *in vitro* and in ovalbumin-induced mouse model. *Biological Research, 49*(41), 1–11.

© COPYRIGHT UPM

Janovik, V., Boligon, A.A., Frohlich, J.K., Schwanz, T.G., Pozzebon, T.V., Alves, S.H., & Athayde, M.L. (2012). Isolation and chromatographic analysis of bioactive triterpenoids from the bark extract of *Cariniana domestica* (Mart) Miers. *Natural Product Research, 26*, 66–71.

Jayavasu, C., Balachandra, K., Sangkitporn, S., Maharongrengrat, A., Thavatsupa, P., Bunjob, M., Chavalittumrong, P., Dechatiwongse, T., & Sittisomwong, N. (1992a). Clinical trial in the treatment of genital herpes patients with *Clinacanthus nutans* extract. *Communicable Disease Journal, 18*(3), 152–161.

Jayavasu, C., Dechatiwongse, T., Balachandra, K., Chavalittumrong, P., & Jongtrakulsiri, S. (1992b). The virucidal activity of *Clinacanthus nutans* Lindau extracts against herpes simplex virus type-2: An *in vitro* study. *Bulletin of the Department of Medical Sciences, 34*(4), 153–158.

Jensen-Jarolim, E., Pali-Schöll, I., & Roth-Walter, F. (2017). Outstanding animal studies in allergy I. From asthma to food allergy and anaphylaxis. *Current Opinion in Allergy and Clinical Immunology, 17*, 169–179.

Jeong, Y., Oh, Y.-C., Cho, W.-K., Lee, B., Ma, J. (2016). Anti-inflammatory effects of Melandrii herba ethanol extract via inhibition of NF-κB and MAPK signaling pathways and Induction of HO-1 in RAW 264.7 cells and mouse primary macrophages. *Molecules, 21*, 818.

Jerman Klen, T., & Vodopivec, B.M. (2012). Optimisation of olive oil phenol extraction conditions using a high-power probe ultrasonication. *Food Chemistry, 134,* 2481–2488.

Ji, H.F., Du, A.L., Zhang, L.W., Xu, C.Y., Yang, M.D. & Li, F.F. (2012). Effects of drying methods on antioxidant properties in *Robinia pseudoacacia* L. flowers. *Journal of Medicinal Plant Research, 6*, 3233–3239.

Jiang, S., Nakano, Y., Rahman, M.A., & Kamei, C. (2008). Effects of a *Dictamnus dasycarpus* T. extract on allergic models in mice. *Bioscience, Biotechnology, and Biochemistry, 72* (3), 660-665.

Joo, T., Sowndhararajan, K., Hong, S., Lee, J., Park, S.Y., Kim, S., & Jhoo, J.W. (2014). Inhibition of nitric oxide production in LPS-stimulated RAW 264.7 cells by stem bark of *Ulmus pumila* L. *Saudi Journal of Biological Sciences, 21*(5), 427–435.

Joycharat, N., Greger, H., Hofer, O., & Saifah, E. (2008). Flavaglines and triterpenoids from the leaves of *Aglaia forbesii*. *Phytochemistry, 69*, 206–211.

Jurikova, T., & Konstant, U. (2015). Polyphenols and their mechanism of action in allergic immune response. *Global Journal of Allergy, 1* (2), 8–11.

© COPYRIGHT UPM

Kaloyianni, M., & Freedland, R.A. (1990). Contribution of several amino acids and lactate to gluconeogenesis in hepatocytes isolated from rats fed various diets. *American Institute of Nutrition, 120*, 116–122.

Kanehisa, M., Araki, M., Goto, S., Hattori, M., Hirakawa, M., Itoh, M., & Yamanishi, Y. (2008). KEGG for linking genomes to life and the environment. *Nucleic Acids Research*, *36*, 480–484.

Kawai, M., Hirano, T., Higa, S., Arimitsu, J., Maruta, M., Kuwahara, Y., Ohkawara, T., Hagihara, K., Yamadori, T., Shima, Y., & Ogata, A. (2007). Flavonoids and related compounds as anti-allergic substances. *Allergology International, 56*, 113-123.

Kelava, T., Cavar, I., & Culo, F. (2011). Biological actions of drug solvent. *Periodicum Biologorum, 113*(3), 311–320.

Kelm, M. (1999). Nitric oxide metabolism and breakdown. *Biochimica et Biophysica Acta (BBA) - Bioenergetics, 1411*(2-3), 273–289.

Kendall, R. V., & Lawson, J. W. (1991). *U.S. Patent No. 5,026,728.* Washington, DC: U.S. Patent and Trademark Office.

Khanam, S., & Sultana, R. (2012). Isolation of *β*-sitosterol & stigmasterol as active immunomodulatory constituents from fruits of *Solanum xanthocarpum* (Solanaceae). *International Journal of Pharmaceutical Sciences and Research, 3*, 9–12.

Khoddami, A., Wilkes, M.A., & Roberts, T.H. (2013). Techniques for analysis of plant phenolic compounds. *Molecules, 18,* 2328–2375.

Kim, D., Lee, S., Kim, W., Jiang, J., Kim, M., Shin, Y., Kim, D., Moon, W., Kwon, S., Koppula, S., Kang, T., & Lee, K. (2012). Inhibitory effects of *Acorus calamus* extracts on mast cell-dependent anaphylactic reactions using mast cell and mouse model. *Journal of Ethnopharmacology, 141*(1), 526–529.

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., Choi, Y.H., & Verpoorte, R. (2010a). NMR-based metabolomic analysis of plants. *Nature Protocols, 5*(3), 536–549.

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

© COPYRIGHT UPM

Kim, H.K., Song, C.H., Bae, Y.S., Im, S.Y., & Lee, H.K. (2016). Glutamine prevents late-phase anaphylaxis via MAPK phosphatase 1-dependent cytosolic phospholipase A_2 deactivation. *International Archives of Allergy and Immunology*, *171,* 61–70

Kim, M., Kim, S.Y., Randy, A., Lim, S.J., Dorjsembe, B., & Nho, C.W. (2016). Inhibitory effect of the *Larix sibirica* and its various flavonoids on the IgE-stimulated mast cell activation and anaphylaxis. *Journal of Functional Foods, 27*, 631–644.

Kirkeboen, K.A., & Strand, O.A. (1999). The role of nitric oxide in sepsis-an overview. *Acta Anaesthesiologica Scandinavica, 43*, 275–288.

Kittisiripornkul, S., & Witthayāsāt, K. (1984). *The antiinflammatory action and toxicological studies of extracts from Clinacanthus nutans* (Unpublished master thesis). Mahidol University, Bangkok, Thailand.

Knipping, K., Van Esch, E.C.A.M., Wijering, S.C., Van Der Heide, S., Dubois, A.E., & Johan, G. (2008). *In vitro* and *in vivo* anti-allergic effects of *Arctium lappa* L. *Experimental Biology and Medicine*, 1469–1477.

Kong, J., Chalcraft, K., Mandur, T.S., Walker, T.D., Goncharova, S., Naji, L., Flader, K., & Larch, M. (2015). Comprehensive metabolomics identifies the alarmin uric acid as a critical signal for the induction of peanut allergy, *European Journal of Allergy and Clinical Immunology, 70,* 495–505.

Kosai, P., Sirisidthi, K., & Jiraungkoorskul, W. (2016). Evaluation of total phenolic compound and cytotoxic activity of *Clinacanthus nutans*. *Indian Journal of Pharmaceutical Sciences, 78*(2), 283–286.

Kumar, D.S. (2015). *Herbal Bioactives and Food Fortification: Extraction and Formulation*. CRC Press, Boca Raton.

Kunsorn, P., Praphasawat, R., Tangnitipong, S., Phuneerub, P., Charoensap, R., & Sapbamrer, R. (2016). *In vitro* safety assessment of *Clinacanthus nutans* crude extracts. *MFUIC & KTCM,* 53–59.

Kunsorn, P., Ruangrungsi, N., Lipipun, V., Khanboon, A., & Rungsihirunrat, K. (2013). The identities and anti-herpes simplex virus activity of *Clinacanthus nutans* and *Clinacanthus siamensis*. *Asian Pacific Journal of Tropical Biomedicine, 3*(4), 284–290.

Kyei, S., Koffuor, G.A., Ramkissoon, P., Abokyi, S., Owusu-afriyie, O., & Wiredu, E.A. (2015). Possible mechanism of action of the antiallergic effect of an aqueous extract of *Heliotropium indicum* L. in ovalbumin-induced allergic conjunctivitis. *Journal of Allergy,* 2015.

© COPYRIGHT UPM

Laffel, L. (1999). Ketone bodies: a review of physiology, pathophysiology and application of monitoring to diabetes. *Diabetes/Metabolism Research and Reviews, 15*(6), 412–426.

Lau, K.W., Lee, S.K., & Chin, J.H., (2014). Effect of the methanol leaves extract of *Clinacanthus nutans* on the activity of acetylcholinesterase in male mice. *Journal of Acute Disease, 3*(1), 22–25.

Lavergne, F.D., Broeckling, C.D., Cockrell, D.M., Haley, S.D., Peairs, F.B., Jahn, C.E., & Heuberger, A.L. (2018). GC-MS metabolomics to evaluate the composition of plant cuticular waxes for four *Triticum aestivum* cultivars. *International Journal of Molecular Sciences, 19*(249), 1–21.

Le, C.F., Kailaivasan, T.H., Chow, S.C., Abdullah, Z., Ling, S.K., & Fang, C.M. (2017). Phytosterols isolated from *Clinacanthus nutans* induce immunosuppressive activity in murine cells. *International Immunopharmacology, 44,* 203–210.

Lee, A.J., Thalayasingam, M., & Lee, B.W. (2013). Food allergy in Asia: how does it compare? *Asia Pacific Allergy, 3*, 3.

Lee, D.Y., Li, H., Lim, H.J., Lee, H.J., Jeon, R., & Ryu, J.H. (2012). Anti-inflammatory activity of sulfur-containing compounds from garlic. *Journal of Medicinal Food, 15*(11), 992–999.

Lee, S.Y., Abas, F., Khatib, A., Ismail, I.S., Shaari, K., & Zawawi, N. (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.

Lee, S.Y., Mediani, A., Nur Ashikin, A.H., Azliana, A.B.S., & Abas, F. (2014). Antioxidant and α-glucosidase inhibitory activities of the leaf and stem of selected traditional medicinal plants. *International Food Research Journal, 21*(1), 165–172.

Leong, S.W., Mohd Faudzi, S.M., Abas, F., Mohd Aluwi, M.F.F., Rullah, K., Wai, L.K., Abdul Bahari, M.N., Ahmad, S., Tham, C.L., Shaari, K., & Lajis, N.H. (2014). Synthesis and sar study of diarylpentanoid analogues as new anti-inflammatory agents. *Molecules, 19*(10), 16058–16081.

Lever, M., & Slow, S. (2010). The clinical significance of betaine, an osmolyte with a key role in methyl group metabolism. *Clinical Biochemistry, 43*(9), 732–744.

Levey, H.A. (1969). Toxicity of the venom of the sea-snake, *Laticauda colubrina*, with observations on a Malay "folk cure". *Toxicon, 6*, 269–276.

Li, M., Wang, J., Lu, Z., Wei, D., Yang, M., & Kong, L. (2014). NMR-based metabolomics approach to study the toxicity of lambda-cyhalothrin to goldfish (*Carassius auratus*). *Aquatic Toxicology, 146*, 82–92.

© COPYRIGHT UPM

Liew, S.Y., Stanbridge, E.J., Yusoff, K., & Shafee, N. (2012). Hypoxia affects cellular responses to plant extracts. *Journal of Ethnopharmacology, 144*(2), 453–456.

Liju, V.B., Jeena, K., & Kuttan, R. (2013). Acute and subchronic toxicity as well as mutagenic evaluation of essential oil from turmeric (*Curcuma longa* L). *Food and Chemical Toxicology, 53*, 52–61.

Lin, C.-M., Huang, S.-T., Liang, Y.-C., Lin, M.-S., Shih, C.-M., Chang, Y.-C., Chen, T.-Y., & Chen, C.-T. (2005). Isovitexin suppresses lipopolysaccharide-mediated inducible nitric oxide synthase through inhibition of NF-kappa B in mouse macrophages. *Planta Medica, 71*(8), 748–753.

Lin, C., Hsieh, S., Wu, C., Huang, G., Leung, W., Lee, T., Lee, C., & Chen, C. (2017). *Polygonum multiflorum* water extract protects against nitric oxide by LPS-induced in RAW 264. 7 macrophages. *Biomedical Research, 28*(6), 2854–2858.

Lin, J., Li, H.M., & Yu, J.G. (1983). Studies on the chemical constituents of niu xu hua (*Clinacanthus nutans*). *Zhongcaoyao, 14*, 337–338.

Lin, Y. Te, Wu, C. Te, Huang, J.L., Cheng, J.H., & Yeh, K.W. (2016). Correlation of ovalbumin of egg white components with allergic diseases in children. *Journal of Microbiology, Immunology and Infection, 49*, 112–118.

Liu, J., Zhang, Q., Liu, M., Ma, L., Shi, Y., & Ruan, J. (2016). Metabolomic analyses reveal distinct change of metabolites and quality of green tea during the shortduration of a single spring season. *Journal of Agricultural and Food Chemistry, 64*(16), 3302–3309.

Liu, N.Q., Cao, M., Frédérich, M., Choi, Y.H., Verpoorte, R., & van der Kooy, F. (2010). Metabolomic investigation of the ethnopharmacological use of *Artemisia afra* with NMR spectroscopy and multivariate data analysis. *Journal of Ethnopharmacology, 128*(1), 230–235.

Liu, P., Kang, X.C., Hu, X., Pan, S.Q., Liu, J.G., Kang, J., Xie, H.Q., Wei, Y.L., Ji, X.L., Zhang, M.H., & Liu, D.B. (2014). Urine metabolic profiling analysis revealed altered metabolic pathways in anaphylactic animals. *Chronic Disease Management and Subhealth Intervention, 1*, 1–13.

Lorke, D. (1983). A new approach to practical acute toxicity testing. A*rchives of Toxicology, 54*(4), 275–287.

Lou, S.N., Hsu, Y.S., & Ho, C.T. (2014). Flavonoid compositions and antioxidant activity of calamondin extracts prepared using different solvents. *Journal of Food and Drug Analysis, 22*(3), 290–295.

© COPYRIGHT UPM

Lusia Barek, M., Hasmadi, M., Zaleha, A.Z., & Mohd Fadzelly, A.B. (2015). Effect of different drying methods on phytochemicals and antioxidant properties of unfermented and fermented teas from Sabah snake grass (*Clinacanthus nutans* Lind.) leaves. *International Food Research Journal, 22*(2), 661–670.

Lv, H., Jiang, F., Guan, D., Lu, C., Guo, B., Chan, C., Peng, S., Liu, B., Guo, W., Zhu, H., Xu, X., Lu, A., & Zhang, G. (2016). Metabolomics and its application in the development of discovering biomarkers for osteoporosis research. *International Journal of Molecular Sciences, 17*(12)*, 1-22.*

Ma, X.H., Zheng, C.J., Han, L.Y., Xie, B., Jia, J., Cao, Z.W., Li, Y.X., & Chen, Y.Z. (2009). Synergistic therapeutic actions of herbal ingredients and their mechanisms from molecular interaction and network perspectives. *Drug Discovery Today, 14*(11-12), 579–588.

Machana, S., Weerapreeyakul, N., Barusrux, S., Thumanu, K., & Tanthanuch, W. (2012). Synergistic anticancer effect of the extracts from *Polyalthia evecta* caused apoptosis in human hepatoma (HepG2) cells. *Asian Pacific Journal of Tropical Biomedicine, 2*(8), 589–596.

Mahajan, S.G., & Mehta, A.A. (2007). Inhibitory action of ethanolic extract of seeds of *Moringa oleifera* Lam. on systemic and local anaphylaxis. *Journal of Immunotoxicology, 4*, 287–294.

Mai, C.W., Yap, K.S.I., Kho, M.T., Ismail, N.H., Yusoff, K., Shaari, K., Chin, S.Y., & Lim, E.S.H. (2016). Mechanisms underlying the anti-inflammatory effects of *Clinacanthus nutans* Lindau extracts: Inhibition of cytokine production and toll-like receptor-4 activation. *Frontiers in Pharmacology, 7*(7), 1–11.

Maiuolo, J., Oppedisano, F., Gratteri, S., Muscoli, C., & Mollace, V. (2016). Regulation of uric acid metabolism and excretion. *International Journal of Cardiology, 213*, 8–14.

Mak (2012). Dimethylamine. *The MAK Collection for Occupational Health and Safety, 7*, 76–89.

Mantle, P.G., Nicholls, A.W., & Shockcor, J.P. (2011). ^{1}H NMR spectroscopy-based metabolomic assessment of uremic toxicity, with toxicological outcomes, in male rats following an acute, mid-life insult from ochratoxin A. *Toxins, 3*(6), 504–519.

Marzocco, S., Di Paola, R., Ribecco, M.T., Sorrentino, R., Domenico, B., Genesio, M., Pinto, A., Autore, G., & Cuzzocrea, S. (2004a). Effect of methylguanidine in a model of septic shock induced by LPS. *Free Radical Research, 38*(11), 1143–1153.

© COPYRIGHT UPM

Marzocco, S., Di Paola, R., Serraino, I., Sorrentino, R., Meli, R., Mattaceraso, G., Cuzzocrea, S., Pinto, A., & Autore, G. (2004b). Effect of methylguanidine in carrageenan-induced acute inflammation in the rats. *European Journal of Pharmacology, 484*, 341–350.

Matés, J.M., Pérez-Gómez, C., & Blanca, M. (2000). Chemical and biological activity of free radical "scavengers" in allergic diseases. *Clinica Chimica Acta, 296*(1-2), 1–15.

Mato, J.M. (1990). *Phospholipid metabolism in cellular signaling*. CRC Press.

Maulidiani, H., Khatib, A., Shaari, K., Abas, F., Shitan, M., Kneer, R., Neto, V., & Lajis, N.H. (2012). Discrimination of three pegaga (Centella) varieties and determination of growth-lighting effects on metabolites content based on the chemometry of ^{1}H nuclear magnetic resonance spectroscopy. *Journal of Agricultural and Food Chemistry, 60*(1), 410–417.

Maulidiani, Abas, F., Khatib, A., Perumal, V., Suppaiah, V., Ismail, A., Hamid, M., Shaari, K., & Lajis, N.H. (2016). Metabolic alteration in obese diabetes rats upon treatment with *Centella asiatica* extract. *Journal of Ethnopharmacology, 180*, 60–69.

Mediani, A., Abas, F., Khatib, A., Maulidiani, H., Shaari, K., Choi, Y.H., & Lajis, N.H. (2012). ^{1}H-NMR-based metabolomics approach to understanding the drying effects on the phytochemicals in *Cosmos caudatus*. *Food Research International, 49*, 763–770.

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

Mediani, A., Abas, F., Maulidiani, M., Khatib, A., Tan, C.P., Safinar, I., Shaari, K., Ismail, A., Lajis, N.H. (2016). Metabolic and biochemical changes in streptozotocin induced obese-diabetic rats treated with *Phyllanthus niruri* extract. *Journal of Pharmaceutical and Biomedical Analysis, 128*, 302–312.

Melo, G.O. De, Muzitano, M.F., Legora-machado, A., Almeida, T.A., Oliveira, D.B. De, Kaiser, C.R., Koatz, V.L.G., & Costa, S.S. (2004). C-glycosyl flavones from the aerial parts of *Eleusine indica* inhibit LPS- induced mouse lung inflammation. *Planta Medica, 71*, 362–363.

Miao, Y.-J., Shi, Y.-Y., Li, F.-Q., Shan, C.-X., Chen, Y., Chen, J.-W., & Li, X. (2016). Metabolomics study on the toxicity of *Annona squamosa* by ultraperformance liquid-chromatography high-definition mass spectrometry coupled with pattern recognition approach and metabolic pathways analysis. *Journal of Ethnopharmacology, 184*, 187–195.

© COPYRIGHT UPM

Middelveld, R.J.M., & Zetterquist, W.C.G., Bergman, D., Alving, K. (2000). Nitric oxide synthase inhibition augments acute allergic reactions in the pig airways *in vivo*. *European Respiratory Journal, 16*, 836–844.

Mie, A., Laursen, K.H., Aberg, K.M., Forshed, J., Lindahl, A., Thorup-Kristensen, K., Olsson, M., Knuthsen, P., Larsen, E.H., & Husted, S. (2014). Discrimination of conventional and organic white cabbage from a long-term field trial study using untargeted LC-MS-based metabolomics. *Analytical and Bioanalytical Chemistry, 406*, 2885–2897.

Ming, Y., Hao, W., Peng, G., & Luyong, Z. (2016). *China Patent No. CN105395542 A*. Shanghai, China: Patent & Trademark Office.

Mitchell, S.C., & Zhang, A.Q. (2001). Methylamine in human urine. *Clinica Chimica Acta, 312*(1-2), 107–114.

Mitsuhata, H., Shimizu, R., & Yokoyama, M.M. (1995). Role of nitric oxide in anaphylactic shock. *Journal of Clinical Immunology, 15*(6), 277-283.

Miyajima, I., Dombrowicz, D., Martin, T.R., Ravetch, J. V., Kinet, J.P., & Galli, S.J. (1997). Systemic anaphylaxis in the mouse can be mediated largely through IgG1 and FcγRIII. *The Journal of Clinical Investigation, 99*(5), 901–914.

Molyneux, P. (2004). The use of the stable free radical diphenylpicryl-hydrazyl (DPPH) for estimating antioxidant activity. *Songklanakarin Journal of Science and Technology, 26*(2), 211–219.

Moreno, J.J. (2003). Effect of olive oil minor components on oxidative stress and arachidonic acid mobilization and metabolism by macrophages RAW 264.7. *Free Radical Biology and Medicine, 35*(9), 1073–1081.

Mosser, D.M., & Zhang, X. (2008). Activation of murine macrophages. *Current Protocols in Immunology,* 1–9.

Moussatch, H., & Prouvost-Danon, A. (1958). Influence of sodium succinate and malonate on the histamine release in the anaphylactic reaction *in vitro*. *Archives of Biochemistry and Biophysics, 77*, 108–111.

Mushtaq, M.Y., Choi, Y.H., Verpoorte, R., & Wilson, E.G. (2014). Extraction for metabolomics: Access to the metabolome. *Phytochemical Analysis, 25*(4), 291–306.

Mustapa, A.N., Martin, A., Mato, R.B., & Cocero, M.J. (2015). Extraction of phytocompounds from the medicinal plant *Clinacanthus nutans* Lindau by microwave-assisted extraction and supercritical carbon dioxide extraction. *Industrial Crops and Products, 74*, 83–94.

© COPYRIGHT UPM

Na-Ayudhaya, T.D., Sakdarat, S., Shuyprom, A., Pattamadilok, D., Bansiddhi, J., Waterman, P.G., & Karagianis, G. (2001). Chemical constituents of the leaves of *Clinacanthus nutans* Lindau. *Thai Journal of Phytopharmacy, 8*(1), 1-8.

Naik, S.R., Bhagat, S., Shah, P.D., Tare, A.A., Ingawale, D., & Wadekar, R.R. (2013). Evaluation of anti-allergic and anti-anaphylactic activity of ethanolic extract of *Zizyphus jujuba* fruits in rodents. *Brazilian Journal of Pharmacognosy, 23*, 811–818.

Nakamura, S., Hisamura, R., Shimoda, S., Shibuya, I., & Tsubota, K. (2014). Fasting mitigates immediate hypersensitivity: A pivotal role of endogenous D-beta-hydroxybutyrate. *Nutrition and Metabolism, 11*(40), 1–10.

Nicholson, J. K., Lindon, J. C., & Holmes, E. (1999). 'Metabonomics': Understanding the metabolic responses of living systems to pathophysiological stimuli via multivariate statistical analysis of biological NMR spectroscopic data. *Xenobiotica, 29*(11), 1181-1189.

Nurulita, Y., Dhanutirto, H., & Soemardji, A.A. (2008). Refining activity and antidiabetic compound leaf aqueous extract Dandang Gendis. *Jurnal Natur Indonesia, 10*(2), 98–103.

Obiri, D.D., Osafo, N., & Abotsi, R.E. (2013). Antiallergic and antiarthritic effects of stem bark extract of *Glyphaea brevis* (Spreng) Monachino (Tiliaceae) in murine models. *ISRN Pharmacology, 2013*, 874263.

Oettgen, H.C., Martin, T.R., Wynshaw-Boris, A., C., D., Drazen, J.M., & Leder, P. (1994). Active anaphylaxis in IgE-deficient mice. *Letters to Nature, 370*, 367–370.

Okunuki, H., Teshima, R., Sakushima, J., Akiyama, H., Goda, Y., Toyoda, M., & Sawada, J. (2000). Induction of active systemic anaphylaxis by oral sensitization with ovalbumin in mast-cell-deficient mice. *Immunology Letters, 74*, 233–237.

Ong, S.L., Paneerchelvan, S., Lai, H.Y., & Rao, N.K., (2014). *In vitro* lipase inhibitory effect of thirty two selected plants in Malaysia. *Asian Journal of Pharmaceutical and Clinical Research, 7*(2), 19–24.

Organisation of Economic Cooperation and Development (OECD) 423 (2001). Guideline for testing of chemicals.

Oshiro, T.T., Teixeira, C.F.P., & Oga, S. (1990). Propylene glycol enhances anti-inflammatory effects of phenylbutazone. *General Pharmacology, 21*(1), 131–134.

© COPYRIGHT UPM

Othman, A.R., Abdullah, N., Ahmad, S., Ismail, I.S., & Zakaria, M.P. (2015). Elucidation of *in vitro* anti-inflammatory bioactive compounds isolated from *Jatropha curcas* L. plant root. *BMC Complementary and Alternative Medicine, 15*(1), 1–10.

Oyoshi, M.K., Oettgen, H.C., Chatila, T.A., Geha, R.S., & Bryce, P.J. (2014). Food allergy: Insights into etiology, prevention, and treatment provided by murine models. *Journal of Allergy and Clinical Immunology, 133*(2), 309–317.

P'ng, X.W., Akowuah, G.A., & Chin, J.H. (2012). Acute oral toxicity study of *Clinacanthus nutans* in mice. *International Journal of Pharmaceutical Sciences and Research, 3*(11), 4202–4205.

Pang, K. A., Pang, K. P., Pang, E. B., Cherilynn, T. Y. N., Chan, Y. H., & Siow, J. K. (2017). Food allergy and allergic rhinitis in 435 asian patients - A descriptive review. *Medical Journal of Malaysia*, *72*(4), 215–220.

Pannangpetch, P., Laupattarakasem, P., Kukongviriyapan, V., Kukongviriyapan, U., Kongyingyoes, B., & Aromdee, C. (2007). Antioxidant activity and protective effect against oxidative hemolysis of *Clinacanthus nutans* (Burm. F) Lindau. *Songklanakarin Journal of Science and Technology, 29*(1), 1–9.

Panyakom, K. (2006). *Structural elucidation of bioactive compounds of Clinacanthus nutans (Burm. F.) Lindau leaves* (Unpublished master thesis). Suranaree University of Technology, Nakhon Ratchasima, Thailand.

Pariyani, R., Ismail, S., Amalina, A.A., Abas, F., & 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, 97*, 4169–4179.

Park, J.-S., Lee, E.-J., Lee, J.-C., Kim, W.-K., & Kim, H.-S. (2007). Anti-inflammatory effects of short chain fatty acids in IFN-γ-stimulated RAW 264.7 murine macrophage cells: involvement of NF-κB and ERK signaling pathways. *International Immunopharmacology, 7*(1), 70–77.

Petterino, C., & Argentino-Storino, A. (2006). Clinical chemistry and haematology historical data in control Sprague-Dawley rats from pre-clinical toxicity studies. *Experimental and Toxicologic Pathology, 57*(3), 213–219.

Phaniendra, A., & Babu, D. (2015). Free radicals : Properties, sources, targets, and their implication in various diseases, *Indian Journal of Clinical Biochemistry, 30*(1), 11–26.

© COPYRIGHT UPM

Pongmuangmul, S., Phumiamorn, S., Sanguansermsri, P., Wongkattiya, N., Fraser, I.H., & Sanguansermsri, D. (2016). Anti-herpes simplex virus activities of monogalactosyl diglyceride and digalactosyl diglyceride from *Clinacanthus nutans*, a traditional Thai herbal medicine. *Asian Pacific Journal of Tropical Biomedicine, 6*(3), 192–197.

Pongphasuk, N., Khunkitti, W., & Chitcharoenthum, M. (2005). Anti-inflammatory and analgesic activities of the extract from *Garcinia mangostana* linn. *Acta Horticulturae, 680*, 125–130.

Prakash, V. (2017). Terpenoids as source of anti-inflammatory compounds. *Asian Journal of Pharmaceutical and Clinical Research, 10*(3), 68.

Prescott, S., & Allen, K.J. (2011). Food allergy: Riding the second wave of the allergy epidemic. *Pediatric Allergy and Immunology, 22*(12), 155–160.

Prior, R.L., Wu, X., & Schaich, K. (2005). Standardized methods for the determination of antioxidant capacity and phenolics in foods and dietary supplements. *Journal of Agricultural and Food Chemistry, 53*, 4290–4302.

Pryde, S.E., Duncan, S.H., Hold, G.L., Stewart, C.S., & Flint, H.J. (2002). The microbiology of butyrate formation in the human colon. *FEMS Microbiology Letters, 217*(2), 133–139.

Purushotham, K., Annegowda, H., Sathish, N., Ramesh, B., & Mansor, S. (2014). Evaluation of phenolic content and antioxidant potnecy in various parts of *Cassia auriculata*. *Pakistan Journal of Biological Sciences, 17*(1), 41–48.

Putwatana, P., Sanmanowong, P., Oonprasertpong, L., Junda, T., Pitiporn, S., & Narkwong, L. (2009). Relief of radiation-induced oral mucositis in head and neck cancer. *Cancer Nursing, 32*(1), 82–87.

Rathnasamy, S., Mohamed, K., Sulaiman, S.F., & Akinboro, A. (2013). Evaluation of cytotoxic , mutagenic and antimutagenic potential of leaf extracts of three medicinal plants using *Allium cepa* chromosome assay. *International Current Pharmaceutical Journal, 2*(8), 131–140.

Raya, K.B., Ahmad, S.H., Farhana, S.F., Mohammad, M., Tajidin, N.E., & Parvez, A. (2015). Changes in phytochemical contents in different parts of *Clinacanthus nutans* (Burm. F.) Lindau due to storage duration. *Bragantia, 74*(4), 445–452.

Reber, L.L., Hernandez, J.D., & Galli, S.J. (2017). The pathophysiology of anaphylaxis. *Journal of Allergy and Clinical Immunology, 140*, 335–348.

Roeslan, M.O., Na Ayudhya, T.D., & Sittichai, K. (2012). Characteristics of *Clinacanthus nutans* extraction from Thailand and Indonesia (preliminary study), in: Conference on Graduate Student Network of Thailand.

© COPYRIGHT UPM

Rolla, G., Nebiolo, F., Guida, G., Heffler, E., Bommarito, L., & Bergia, R. (2006). Case report level of exhaled nitric oxide during human anaphylaxis. *Annals of Allergy, Asthma and Immunology, 97*(2), 264–265.

Roosita, K., Kusharto, C.M., Sekiyama, M., Fachrurozi, Y., & Ohtsuka, R. (2008). Medicinal plants used by the villagers of a Sundanese community in West Java, Indonesia. *Journal of Ethnopharmacology, 115*(1), 72–81.

Sade, K., Schwartz, D., Wolman, Y., Schwartz, I., Chernichovski, T., Blum, M., Brazowski, E., Keynan, S., Raz, I., Blantz, R.C., & Iaina, A. (1999). Time course of lipopolysaccharide-induced nitric oxide synthase mRNA expression in rat glomeruli. *Journal of Laboratory and Clinical Medicine,* 471–477.

Sade, K., Schwartz, I.F., Etkin, S., Schwartzenberg, S., Levo, Y., & Kivity, S. (2007). Expression of inducible nitric oxide synthase in a mouse model of anaphylaxis. *Journal of Investigational Allergology and Clinical Immunology, 17*(6), 379–385.

Sakdarat, S., Shuyprom, A., Dechatiwongse, N.A.T., Waterman, P.G., & Karagianis, G. (2006). Chemical composition investigation of the *Clinacanthus nutans* Lindau leaves. *Thai Journal of Phytopharmacy, 13*(2), 13–24.

Sakdarat, S., Shuyprom, A., Pientong, C., Ekalaksananan, T., & Thongchai, S. (2009). Bioactive constituents from the leaves of *Clinacanthus nutans* Lindau. *Bioorganic and Medicinal Chemistry, 17*(5), 1857–1860.

Salek, R., Emery, L., & Beisken, S. (2014). Metabolomics: An introduction. Retrieved from [illegible]

Sangkitporn, S., Chaiwat, S., Balachandra, K., Dechatiwongse Na-Ayudhaya, T., Bunjob, M., & Jayavasu, C. (1995). Treatment of herpes zoster with *Clinacanthus nutans* (Bi Phaya Yaw) extract. *Journal of the Medical Association of Thailand, 78*(11), 624–627.

Sangkitporn, S., Kroavon, B., Thawatsupha, P., Bunjob, M., & Chavalittumrong, P. (1993). Treatment of recurrent genital herpes simplex virus infection with *Clinacanthus nutans* extract. *Bulletin of the Department of Medical Service, 18*, 226–231.

Sanjivkumar, B., Usha, G., Mallikarjun, M., & Rao, K.S. (2011). Isolation of 12, 13-dihydro-20, 20-dihydro lupeol from the aqueous extract of *Aesculus hippocastanum* seeds. *International Research Journal of Pharmacy, 2*, 231–233.

Santoro, D., & Marsella, R. (2014). Animal models of allergic diseases. *Veterinary Sciences, 1*, 192–212.

© COPYRIGHT UPM

Sarega, N., Imam, M.U., Md Esa, N., Zawawi, N., & Ismail, M. (2016a). Effects of phenolic-rich extracts of *Clinacanthus nutans* on high fat and high cholesterol diet-induced insulin resistance. *BMC Complementary and Alternative Medicine, 16*(1), 88.

Sarega, N., Imam, M.U., Ooi, D.J., Chan, K.W., Md Esa, N., Zawawi, N., & Ismail, M. (2016b). Phenolic rich extract from *Clinacanthus nutans* attenuates hyperlipidemia-associated oxidative stress in rats. *Oxidative Medicine and Cellular Longevity, 2016*, 4137908.

Satakhun, S. (2001). Chemical constituents of *Clinacanthus nutans* leaves. *Thai Journal of Pharmaceutical Science*, *25*, 38.

Satatyavivad, J., Bunyaprephatsara, N., Kitisiripornkul, S., & Tanasomwang, W. (1996). Analgesic and anti-inflammatory activities of extract of *Clinacanthus nutans* (Burm.F.) Lindau. *Thai Journal of Pharmceutical Sciences, 3*(1), 7–17.

Saude, E.J., Skappak, C.D., Regush, S., Cook, K., Ben-Zvi, A., Becker, A., Moqbel, R., Sykes, B.D., Rowe, B.H., & Adamko, D.J. (2011). Metabolomic profiling of asthma: Diagnostic utility of urine nuclear magnetic resonance spectroscopy. *Journal of Allergy and Clinical Immunology, 127*(3), 757–764.

Sawada, Y., Akiyama, K., Sakata, A., Kuwahara, A., Otsuki, H., Sakurai, T., & Hirai, M. Y. (2009). Widely targeted metabolomics based on large-scale MS/MS data for elucidating metabolite accumulation patterns in plants. *Plant and Cell Physiology*, *50*(1), 37-47.

Scalbert, A., Brennan, L., Fiehn, O., Hankemeier, T., Kristal, B.S., van Ommen, B., Pujos-Guillot, E., Verheij, E., Wishart, D., & Wopereis, S. (2009). Mass-spectrometry-based metabolomics: Limitations and recommendations for future progress with particular focus on nutrition research. *Metabolomics, 5*, 435–458.

Sejali, S.N.F., & Anuar, M.S. (2011). Effect of drying methods on phenolic contents of neem (*Azadirachta indica*) leaf powder. *Journal of Herbs, Spices & Medicinal Plants, 17*(2), 119–131.

Sekar, M., & Rashid, N.A. (2016). Formulation, evaluation and antibacterial properties of herbal ointment containing methanolic extract of *Clinacanthus nutans* leaves. *International Journal of Pharmaceutical and Clinical Research, 8*(8), 1170–1174.

Sharma, S. (2011). *Plant taxonomy* (Second). New Delhi: Pacific Book International.

Shim, S.Y., Aziana, I., & Khoo, B.Y. (2013). Perspective and insight on *Clinacanthus nutans* Lindau in traditional medicine. *International Journal of Integrative Biology, 14*(1), 7–9.

© COPYRIGHT UPM

Shuyporm, A. (2004). Chemical composition investigation of the *Clinacanthus nutans* (Burm. F.) Lindau leaves. Suranaree University of Technology.

Sicherer, S.H., & Sampson, H.A. (2015). Food allergy : Epidemiology, pathogenesis, diagnosis, and treatment. *Journal of Allergy and Clinical Immunology, 133*(2), 291–307.e5.

Siddhuraju, P., & Becker, K. (2003). Antioxidant properties of various solvent extracts of total phenolic constituents from three different agroclimatic origins of drumstick tree (*Moringa oleifera* Lam.) leaves. *Journal of Agricultural and Food Chemistry, 51*(8), 2144–2155.

Siddique, N.A., Mujeeb, M., Najmi, A.K., Khan, H.N., & Farooqi, H. (2010). Evaluation of antioxidant activity, quantitative estimation of phenols and flavonoids in different parts of *Aegle marmelos*. *Journal of Saudi Chemical Society, 4*(1), 1–5.

Siew, Y.Y., Zareisedehizadeh, S., Seetoh, W.G., Neo, S.Y., Tan, C.H., & Koh, H.L. (2014). Ethnobotanical survey of usage of fresh medicinal plants in Singapore. *Journal of Ethnopharmacology, 155*(3), 1450–1466.

Silverstein, R. M., Webster, F. X., Kiemle, D. J., & Bryce, D. L. (2014). *Spectrometric Identification of Organic Compounds*. John Wiley & Sons.

Simons, F.E.R., Ardusso, L.R.F., & Bilò, M.B. (2011). World allergy organization guidelines for the assessment and management of anaphylaxis. *World Allergy Organization Journal*, 13–37.

Siti Zulaikha, A.G., Mediani, A., Khoo, L.W., Lee, S.Y., Leong, S.W., & Abas, F. (2017). Effect of different drying methods and solvent ratios on biological activities of *Phyllantus acidus* extracts. *International Food Research Journal, 24*(1), 114–120.

Sittiso, S., Ekalaksananan, T., Pientong, C., Sakdarat, S., Charoensri, N., & Kongyingyoes, B. (2010). Effects of compounds from *Clinacanthus nutans* on dengue virus type 2 infection. *Srinagarind Medical Journal, 25*, 272–275.

Smith, A., Coupland, G., Dolan, L., Harberd, N., Jones, J., Martin, C., Sablowski, R., & Amey, A. (2009). *Plant biology*. Garland Science, New York.

Stokol, T. (2013). Veterinary clinical pathology. Cornell university college of veterinary medicine eClinPath the on-line textbook website. Retrieved from http://www.eclinpath.com/

Sofowora, A., Ogunbodede, E., & Onayade, A. (2013). The role and place of medicinal plants in the strategies for disease prevention. *African Journal of Traditional, Complementary and Alternative Medicines, 10*(5), 210–229.

© COPYRIGHT UPM

Son, H.-S., Hwang, G.-S., Kim, K.M., Ahn, H.-J., Park, W.-M., Van Den Berg, F., Hong, Y.-S., & Lee, C.-H. (2009). Metabolomic studies on geographical grapes and their wines using ^{1}H-NMR analysis coupled with multivariate statistics. *Journal of Agricultural and Food Chemistry, 57*(4), 1481–1490.

Sriwanthana, B., Chavalittumrong, P., & Chompuk, L. (1996). Effect of *Clinacanthus nutans* on human cell-mediated immune response *in vitro*. *Thailand Journal of Pharmaceutical Science, 20*(4), 261–267.

Sultana, B., Anwar, F., & Ashraf, M. (2009). Effect of extraction solvent/technique on the antioxidant activity of selected medicinal plant extracts. *Molecules, 14,* 2167–2180.

Suntararuks, S. (1999). *The study of immunotoxic effect of Clinacanthus nutans extract in male wistar rats* (Unpublished master thesis) . Mahidol University, Thailand.

Suzuki, M., Nakamura, T., Iyoki, S., Fujiwara, A., Watanabe, Y., Mohri, K., Isobe, K., Ono, K., & Yano, S. (2005). Elucidation of anti-allergic activities of curcumin-related compounds with a special reference to their anti-oxidative activities. B*iological & Pharmaceutical Bulletin, 28*(8), 1438–43.

Syarina, P.N.A., Karthivashan, G., Abas, F., Arulselvan, P., & Fakurazi, S. (2015). Wound healing potential of *Spirulina platensis* extracts on human dermal fibroblast cells. *EXCLI journal*, *14*, 385.

Tan, C.S.H., Ho, C.F.Y., Heng, S.S., Wu, J.S., Tan, B.K.H., Ng, Y.K., Sun, G.Y., Lin, T.N., & Ong, W.Y. (2016). *Clinacanthus nutans* extracts modulate epigenetic link to cytosolic phospholipase A_2 expression in SH-SY5Y cells and primary cortical neurons. *Neuromolecular Medicine, 18*(3), 441–452.

Tang, R., Xu, H.Y., Cao, J., Chen, S., Sun, J.L., Hu, H., Li, H.C., Diao, Y., & Li, Z. (2015). Clinical characteristics of inpatients with anaphylaxis in China. *BioMed Research International, 2015*, 1–6.

Tedelind, S., Westberg, F., Kjerrulf, M., & Vidal, A. (2007). Anti-inflammatory properties of the short-chain fatty acids acetate and propionate: A study with relevance to inflammatory bowel disease. *World Journal of Gastroenterology, 13*(20), 2826–2832.

Teoh, P.L., Angelina Cheng, Y.F., Liau, M., Lem, F.F., Grace P, K., Chua, F.N., Cheong, B.E. (2017). Chemical composition and cytotoxic properties of *Clinacanthus nutans* root extracts. *Pharmaceutical Biology, 55*(1), 394–401.

Teshima, K., Kaneko, T., Ohtani, K., Kasai, R., Lhieochaiphant, S., Picheansoonthon, C., & Yamasaki, K. (1997). C-glycosyl flavones from *Clinacanthus nutans*. *Natural Medicine Note, 51*(6), 557.

© COPYRIGHT UPM

Teshima, K., Kaneko, T., Ohtani, K., Kasai, R., Lhieochaiphant, S., Picheansoonthon, C., & Yamasaki, K. (1998). Sulfur-containing glucosides from *Clinacanthus nutans*. *Phytochemistry, 48*(5)*,* 831–835.

Thamlikitkul, V. (1995). Treatment of herpes genitalis and herpes zoster with *Clinacanthus nutans*. *Journal of Infectious Diseases and Antimicrobial Agents, 12,* 95–96.

Thawaranantha, D., Balachandra, K., Jongtrakulsiri, S., Chavalittumrong, P., Bhumiswasdi, J., & Jayavasu, C. (1992). *In vitro* antiviral activity of *Clinacanthus nutans* on varicella-zoster virus. *Siriraj Hospital Gazette, 44*(4)*,* 285–291.

Thongchai, S., Ekalaksananan, T., Pientong, C., Aromdee, C., Kongyingyoes, B., & Seubsasana, S. (2008). Anti-herpes simplex virus type 1 activity of crude ethyl acetate extract of *Clinacanthus nutans*. *Journal of Science and Technology Mahasarakham University, 27*(4), 318–326.

Thongharb, C., & Tejasen, P. (1977). The effect of Slaed Pang Porn (*Clinacanthus nutans*) on Thailand cobra venom (*Naja naja siamensis*). *Thai Journal of Pharmaceutical Sciences, 2*, 1057–1063.

Thongrakard, V., & Tencomnao, T. (2010). Modulatory effects of Thai medicinal plant extract on proinflammatory cytokines-induced apoptosis in human keratinocyte HaCaT cells. *African Journal of Biotechnology, 9*(3), 4999–5003.

Thouri, A., Chahdoura, H., El Arem, A., Omri Hichri, A., Ben Hassin, R., & Achour, L. (2017). Effect of solvents extraction on phytochemical components and biological activities of Tunisian date seeds (var. Korkobbi and Arechti). *BMC Complementary and Alternative Medicine, 17*(1), 1–10.

Tiew, W.P., Wen, P.X., Chin, J.H., & Akowuah, G.A. (2014). Effect of methanol extract of *Clinacanthus nutans* on serum biochemical parameters in rats. *Journal of Applied Pharmacy, 6*(1), 77–86.

Timpawat, S., & Vajrabhaya, L. (1994). Clinical evaluation of *Clinacanthus nutans* Lindau in orabase in the treatment of recurrent aphthous stomatitis. *Mahidol Dental Journal, 14*(1), 10–16.

Tinh, T.D.D. (2014). *Biological activities of Clinacanthus nutans (Burm. F) Lindau extracts* (Unpublished bachelor degree thesis). Vietnam National University Ho Chi Minh City, Hanoi, Vietnam.

Tsai, H. Da, Wu, J.S., Kao, M.H., Chen, J.J., Sun, G.Y., Ong, W.Y., & Lin, T.N., (2016). *Clinacanthus nutans* protects cortical neurons against hypoxia-induced toxicity by downregulating HDAC1/6. *Neuromolecular Medicine, 18*(3), 274–282.

© COPYRIGHT UPM

Tsikas, D. (2007). Analysis of nitrite and nitrate in biological fluids by assays based on the Griess reaction : Appraisal of the Griess reaction in the L-arginine/ nitric oxide area of research. *Journal of Chromatography B, 851*, 51–70.

Tu, S.F., Liu, R.H., Cheng, Y. Bin, Hsu, Y.M., Du, Y.C., El-Shazly, M., Wu, Y.C., & Chang, F.R. (2014). Chemical constituents and bioactivities of *Clinacanthus nutans* aerial parts. *Molecules, 19*, 20382–20390.

Tuntiwachwuttikul, P., Pootaeng-On, Y., Phansa, P., & Taylor, W.C. (2004). Cerebrosides and a monoacylmonogalactosylglycerol from *Clinacanthus nutans*. *Chemical & Pharmaceutical Bulletin, 52*(1), 27–32.

Tyagi, R., Rana, P., Gupta, M., Khan, A.R., Devi, M.M., Bhatnagar, D., Roy, R., Tripathi, R.P., & Khushu, S. (2012). Urinary metabolomic phenotyping of nickel induced acute toxicity in rat: An NMR spectroscopy approach. *Metabolomics, 8*(5), 940–950.

Uawonggul, N., Chaveerach, A., Thammasirirak, S., Arkaravichien, T., Chuachan, C., & Daduang, S. (2006). Screening of plants acting against *Heterometrus laoticus* scorpion venom activity on fibroblast cell lysis. *Journal of Ethnopharmacology, 103*(2), 201–207.

Uawonggul, N., Thammasirirak, S., Chaveerach, A., Chuachan, C., Daduang, J., & Daduang, S. (2011). Plant extract activities against the fibroblast cell lysis by honey bee venom. *Journal of Medicinal Plants Research, 5*(10), 1978–1986.

Vajrabhaya, L.O., & Korsuwannawong, S. (2016). Cytotoxicity evaluation of *Clinacanthus nutans* through dimethylthiazol diphenyltetrazolium bromide and neutral red uptake assays. *European Journal of Dentistry, 10*(1), 134–138.

Van Gramberg, J.L., de Veer, M.J., O'Hehir, R.E., Meeusen, E.N.T., & Bischof, R.J. (2013). Use of animal models to investigate major allergens associated with food allergy. *Journal of Allergy, 2013*, 1–10.

Vince, Ö., & Zoltán, M. (2011). *Plant physiology*. Retrieved from https://www.tankonyvtar.hu/en/tartalom/tamop425/0010_1A_Book_angol_01_novenyelettan/index.html

Vinolo, M.A.R., Rodrigues, H.G., Nachbar, R.T., & Curi, R. (2011). Regulation of inflammation by short chain fatty acids. *Nutrients, 3*(10), 858–876.

Voet, D., Voet, J. (2010). Biochemistry, fourth. ed. John Wiley & Sons, USA.

Vongsak, B., Sithisarn, P., Mangmool, S. (2013). Maximizing total phenolics , total flavonoids contents and antioxidant activity of *Moringa oleifera* leaf extract by the appropriate extraction method. *Industrial Crops & Products, 44*, 566–571.

© COPYRIGHT UPM

Wagner, H. (2011). Synergy research: Approaching a new generation of phytopharmaceuticals. *Fitoterapia, 82*(1), 34–37.

Wang, M., Chen, L., Liu, D., Chen, H., Tang, D.D., & Zhao, Y.Y. (2017). Metabolomics highlights pharmacological bioactivity and biochemical mechanism of traditional Chinese medicine. *Chemico-Biological Interactions, 273*, 133–141.

Wanikiat, P., Panthong, A., Sujayanon, P., Yoosook, C., Rossi, A.G., & Reutrakul, V. (2008). The anti-inflammatory effects and the inhibition of neutrophil responsiveness by *Barleria lupulina* and *Clinacanthus nutans* extracts. *Journal of Ethnopharmacology, 116*(2), 234–244.

Waszkowiak, K., & Gliszczyńska-Świgło, A. (2016). Binary ethanol–water solvents affect phenolic profile and antioxidant capacity of flaxseed extracts. *European Food Research and Technology, 242*(5), 777–786.

Westerhuis, J.A., van Velzen, E.J.J., Hoefsloot, H.C.J., & Smilde, A.K. (2010). Multivariate paired data analysis: Multilevel PLSDA versus OPLSDA. *Metabolomics, 6*(1), 119–128.

Wheelock, Å.M., & Wheelock, C.E. (2013). Trials and tribulations of 'omics data analysis: Assessing quality of SIMCA-based multivariate models using examples from pulmonary medicine. *Molecular BioSystems, 9*(11), 2589.

William, C.M. (1927). The oral administration of epinephrine. *Archives of Internal Medicine, 40*(5), 701–714.

Wishart, D. S., Tzur, D., Knox, C., Eisner, R., Guo, A. C., Young, N., Cheng, D., Jewell, K., Arndt, D., Sawhney, S., Fung, C., Mikolai, L., Lewis, M., Coutouly, M.A., Forsythe, I., Tang, P., Shrivastava, S., Jeroncic, K., Stothard, P.,Amegbey, G., Block, D., Hau D.D., Wagner, J., Miniaci, J., Clements, M., Gebremedhin, M., Guo, N., Zhang, Y., Duggan, G.E., Maclnnis, G.D., Welkie, A.M., Dowlatabadi, R., Bamforth, F., Clive, D., Freiner, R.,Li, L., Marrie, T., Sykes, B.D., Vogel, H.J., & Querengesser, L. (2007). HMDB: The human metabolome database. *Nucleic Acids Research, 35* (suppl.1), 521-526.

Wong, F.C., Yong, A.L., Ong, H.C., & Chai, T.T. (2013). Evaluation of the antibacterial activities of selected medicinal plants and determination of their phenolic constituents. *Scienceasia, 39*(6), 591–595.

Wong, F.C., Yong, A.L., Ting, E.P.S., Khoo, S.C., Ong, H.C., & Chai, T.T. (2014). Antioxidant, metal chelating, anti-glucosidase activities and phytochemical analysis of selected tropical medicinal plants. *Iranian Journal of Pharmaceutical Research, 13*(4), 1407–1413.

Worley, B., & Powers, R. (2013). Multivariate analysis in metabolomics. *Current Metabolomics, 1*(1), 92–107.

© COPYRIGHT UPM

Wu, J.S., Kao, M.H., Tsai, H. Da, Cheung, W.M., Chen, J.J., Ong, W.Y., Sun, G.Y., & Lin, T.N. (2017). *Clinacanthus nutans* mitigates neuronal apoptosis and ischemic brain damage through augmenting the C/EBPβ-driven PPAR-γ transcription. *Molecular Neurobiology,* 1–14.

Xiao, Q., Qu, Z., Zhao, Y., Yang, L., & Gao, P. (2017). Orientin ameliorates LPS-induced inflammatory responses through the inhibitory of the NF-*k*B pathway and NLRP3 inflammasome. *Evidence-based Complementary and Alternative Medicine, 2017*, 1–9.

Xu, Y., Dou, D., Ran, X., Liu, C., & Chen, J. (2015). Integrative analysis of proteomics and metabolomics of anaphylactoid reaction induced by Xuesaitong injection. *Journal of Chromatography A, 1416*, 103–111.

Yadav, A., & Naidu, R. (2015). Clinical manifestation and sensitization of allergic children from Malaysia. *Asia Pacific allergy, 5*(2), 78–83.

Yang, J., Zhao, X., Lu, X., Lin, X., & Xu, G. (2015). A data preprocessing strategy for metabolomics to reduce the mask effect in data analysis. *Frontiers in Molecular Biosciences 2*(4), 1–9.

Yingngam, B., Monschein, M., & Brantner, A. (2014). Ultrasound-assisted extraction of phenolic compounds from *Cratoxylum formosum* ssp. formosum leaves using central composite design and evaluation of its protective ability against H_2O_2-induced cell death. *Asian Pacific Journal of Tropical Medicine, 7*(S1), S497–S505.

Yong, Y.K., Tan, J.J., Teh, S.S., Mah, S.H., Ee, G.C.L., Chiong, H.S., Ahmad, Z., Yong, Y.K., Tan, J.J., Teh, S.S., Mah, S.H., Ee, G.C.L., Chiong, H.S., & Ahmad, Z. (2013). *Clinacanthus nutans* extracts are antioxidant with antiproliferative effect on cultured human cancer cell lines. *Evidence-based Complementary and Alternative Medicine, 2013*, 1–8.

Yoo, W., Kim, D., Yoon, H., & Ryu, S. (2017). Enzyme IIA Ntr regulates *Salmonella* invasion via 1,2-propanediol and propionate catabolism. *Scientific Reports, 7*(44827), 1–13.

Yoosook, C., Panpisutchai, Y., Chaichana, S., Santisuk, T., & Reutrakul, V. (1999). Evaluation of anti-HSV-2 activities of *Barleria lupulina* and *Clinacanthus nutans*. *Journal of Ethnopharmacology, 67*(2), 179–187.

Yu, Q., Duan, Z. H., Duan, W. W., Shang, F. F., & Yang, G. X. (2015, December). Analysis and evaluation of nutrition composition of *Clinacanthus nutans*. In *Resources, Environment and Engineering II: Proceedings of the 2nd Technical Congress on Resources, Environment and Engineering (CREE 2015, Hong Kong, 25-26 September 2015)* (p. 369). CRC Press.

© COPYRIGHT UPM

Yuann, J.M.P., Wang, J.S., Jian, H.L., Lin, C.C., & Liang, J.Y. (2012). Effects of *Clinacanthus nutans* (Burm. f) Lindau leaf extracts on protection of plasmid DNA from riboflavin photoreaction. *MC-Transaction on Biotechnology, 4*(1), 45–58.

Zhang, L., Hu, J.J., Lin, J.W., Fang, W.S., & Du, G.H. (2009). Anti-inflammatory and analgesic effects of ethanol and aqueous extracts of *Pterocephalus hookeri* (C.B. Clarke) Höeck. *Journal of Ethnopharmacology, 123*(3), 510–514.

Zhang, Q., Zhang, J., Shen, J., Silva, A., Dennis, D.A., & Barrow, C.J. (2006). A simple 96-well microplate method for estimation of total polyphenol content in seaweeds. *Journal of Applied Phycology, 18*(3-5), 445–450.

Zhang, Y.N., Hu, H.J., Shi, Y.F., Yang, X.Z., Cao, L.G., Wu, J., Asweto, C.O., Feng, L., Duan, J.C., & Sun, Z.W. (2017). ^{1}H NMR-based metabolomics study on repeat dose toxicity of fine particulate matter in rats after intratracheal instillation. *Science of the Total Environment, 589*, 212–221.

Zheng, H., Pan, L., Xu, P., Zhu, J., Wang, R., & Zhu, W. (2017). An NMR-based metabolomic approach to unravel the preventive effect of water-soluble extract from *Dendrobium officinale* kimura & migo on streptozotocin-induced diabetes in mice. *Molecules*, *22*(1543), 1-14.

Zulkipli, I.N., Rajabalaya, R., Idris, A., Sulaiman, N.A., & David, S.R. (2017). *Clinacanthus nutans* : A review on ethnomedicinal uses, chemical constituents and pharmacological properties. *Pharmaceutical Biology, 55*(1), 1093–1113.

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