UNIVERSITI PUTRA MALAYSIA

PHYTOCHEMISTRY OF Calophyllum andersonii AND Calophyllum wallichianum AND THEIR ANTIBACTERIAL ACTIVITIES

TEE KENG HONG

FS 2018 45

PHYTOCHEMISTRY OF *Calophyllum andersonii* AND *Calophyllum wallichianum* AND THEIR ANTIBACTERIAL ACTIVITIES

By

TEE KENG HONG

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

April 2018

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the Degree of Master of Science

PHYTOCHEMISTRY OF *Calophyllum andersonii* AND *Calophyllum wallichianum* AND THEIR ANTIBACTERIAL ACTIVITIES

By

TEE KENG HONG

April 2018

Chairman: Professor Gwendoline Ee Cheng Lian, PhD
Faculty: Science

Plants from the genus *Calophyllum* are known for their rich content of secondary metabolites, especially phenolic compounds such as coumarins, xanthones and flavonoids. Many of these compounds are identified to be the main contributors of the medicinal properties of the plants. Even today, human are still heavily relying on the natural resources to develop new drugs and medicines. The threats of existing and emerging diseases are to be managed by discovering new lead compounds. Natural product research is one of the various approaches to this. This research project aimed to isolate antibacterial compounds which can lead to the discovery of new antibacterial drug candidates for future drug discovery research.

Phytochemical studies have been carried out on the stem bark of two selected plant species, *Calophyllum andersonii* and *Calophyllum wallichianum*. A total of five xanthones and one terpene were isolated from the hexane and chloroform extracts of *Calophyllum andersonii*. The terpene is friedelin **(79)** while the xanthones are macluraxanthone **(74)**, pyranajacareubin **(75)**, calaxanthone I **(76)**, caloxanthone C **(77)**, and euxanthone **(78)**. All of the compounds were isolated for the first time from the plant. Meanwhile, two coumarins and two terpenes were afforded from the hexane and chloroform extracts of *Calophyllum wallichianum*. The terpenes are friedelin **(79)** and stigmasterol **(82)** while the coumarins are wallimarin T **(80)** and calanolide E **(81)**. Wallimarin T **(80)** is a new coumarin. The structures of the isolated

compounds were identified using spectroscopic methods such as MS, IR and NMR.

The plant extracts and two isolated compounds were examined for their antibacterial activities. The samples were tested against four bacterial strains, namely *Bacillus cereus*, *B. megaterium*, *B. pumilus* and *B. subtilis*. The tests conducted include disc diffusion test, minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) assay. In the disc diffusion test, wallimarin T **(80)**, calanolide E **(81)**, the hexane and chloroform extracts of both plants were tested. All of the samples showed activities against the bacteria, expect wallimarin T **(80)**.

The samples that showed activities were then further tested for their MIC values. The plant extracts showed moderate inhibition against all four bacteria, with MIC values ranging from 0.156 to 1.25 mg/ml. The chloroform extract of *Calophyllum andersonii* showed significant inhibition on *B. pumilus* (MIC = 0.039 mg/ml) while calanolide E **(81)** showed weak inhibition on all the bacteria with MIC value ranging from 0.25 to 0.5 mg/ml. The extracts of *Calophyllum wallichianum* showed high bactericidal concentrations ranging from 2.5 to 10 mg/ml. Extracts of *Calophyllum andersonii* recorded lower MBC value compared to extracts of *Calophyllum wallichianum*, ranging from 0.039 to 1.25 mg/ml. Meanwhile, calanolide E **(81)** did not show any bactericidal activity.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk Ijazah Master Sains

FITOKIMIA DARIPADA *Calophyllum andersonii* DAN *Calophyllum wallichianum* DAN AKITIVITI ANTIBAKTERIA MEREKA

Oleh

TEE KENG HONG

April 2018

Pengerusi: Profesor Gwendoline Ee Cheng Lian, PhD
Fakulti: Sains

Tumbuh-tumbuhan dari genus *Calophyllum* dikenali untuk kekayaan kandungan metabolit sekunder mereka, terutamanya sebatian fenolik seperti xanton, koumarin dan flavonoid. Kebanyakan sebatian tersebut merupakan penyebab kepada sifat perubatan tumbuhan ini. Kini, manusia tetap bergantung kepada sumber semulajadi dalam proses pencarian ubat-ubatan baru. Penemuan ubat-ubatan baru amat penting untuk menentang penyakit-penyakit yang baru dan lama. Kajian sebatian semulajadi merupakan salah satu pendekatan kepada ini. Kajian ini bertujuan untuk mencari sebatian antibakteria yang dapat membantu dalam penemuan ubatan antibakteria yang baru untuk kajian pencarian ubat-ubatan dalam masa yang akan datang.

Kajian fitokimia telah dijalankan ke atas kulit pokok dua spesies yang terpilih, iaitu *Calophyllum andersonii* dan *Calophyllum wallichianum*. Sebanyak lima sebatian xanton dan satu sebatian terpenoid telah dijumpai daripada ekstrak heksana dan kloroform *Calophyllum andersonii*. Sebatian xanton tersebut adalah makluraxanton **(74)**, piranojakareubin **(75)**, kaloxanton I **(76)**, kaloxanton C **(77)** dan euxanton **(78)** manakala sebatian terpenoid yang dijumpai adalah friedelin **(79)**. Selain itu, sebanyak dua coumarin dan dua terpenoid telah didapati daripada ekstrak heksana dan kloroform *Calophyllum wallichianum*. Sebatian terpenoid yang didapati adalah friedelin **(79)** dan stigmasterol **(82)**. Sebatian koumarin pula adalah walimarin T **(80)** dan kalanolida E **(81)**. Walimarin T **(80)** merupakan sebatian koumarin yang baru. Kesemua sebatian yang didapati telah dianalisis dengan menggunakan kaedah spektroskopi termasuk MS, IR dan NMR.

Ekstrak dan dua sebatian yang didapati telah diuji untuk sifat anti-bakteria mereka. Sampel yang terpilih telah diuji dengan bakteria termasuk *Bacillus cereus*, *B. megaterium*, *B. pumilus* dan *B. subtilis*. Ujian yang dijalankan termasuk ujian cakera diffusi, MIC dan juga MBC. Dalam ujian cakera diffusi, walimarin T **(80)**, kalanolida E **(81)**, ekstrak heksana dan kloroform kedua-dua pokok telah diuji. Kesemua sampel menunjukkan aktiviti terhadap bakteria tersebut, kecuali walimarin T **(80)**.

Sampel yang menunjukkan aktiviti telah diuji selanjutnya untuk nilai MIC and MBC mereka. Secara kasarnya, ekstrak yang diuji menunjukkan perencatan yang sederhana terhadap bakteria-bakteria yang digunakan dengan nilai MIC antara 0.156 dan 1.25 mg/ml. Ekstrak kloroform *Calophyllum andersonii* menunjukkan perencatan yang kuat terhadap *B. pumilus* dengan nilai MIC di 0.039 mg/ml. Kalanolida E **(81)** menunjukkan perencatan yang lemah dengan nilai MIC di antara 0.25 dan 0.5 mg/ml. Ekstrak heksana dan kloroform *Calophyllum wallichianum* yang diuji menunjukkan nilai MBC yang tinggi di antara 2.5 dan 10 mg/ml. Sementara itu, ekstrak *Calophyllum andersonii* menunjukkan nilai MBC di antara 0.039 ke 1.25 mg/ml. Kalanolida E **(81)** tidak menunjukkan sebarang sifat bakterisidal terhadap kesemua bakteria yang diuji.

ACKNOWLEDGEMENTS

First of all, I would like to express my gratitude towards my supervisor, Prof. Dr. Gwendoline Ee Cheng Lian. I wish to thank her for being so supportive and patience in guiding me throughout the entire project. I believe that everything that I have learnt from her will be useful to me for a lifetime.

Next, I would like to thank my seniors and labmates, Dr. Nor Hisam Zamakhshari, Dr. Thiruventhan Karunakaran, Mr Lee Kar Wei, Mr Wong Ka Woong and Dr Teh Soek Sin. Also, I wish to thank my friend, Mr Laimy for his encouragement. They have been really helpful in my time of needs. They also made my postgraduate journey an interesting and cheerful one. Other than that, I would like to thank the staff and lab technicians of the Department of Chemistry, UPM for their assistance along the way.

Then, I would want to thank the loves of my life, whom are my parents, my family and Qi Ting for being there for me all the time. They have been supportive in every way. Not any of this is possible without them.

All in all, thanks to everyone who helped me in becoming a better person.

I certify that a Thesis Examination Committee has met on 24 April 2018 to conduct the final examination of Tee Keng Hong on his thesis entitled "Phytochemistry of *Calophyllum andersonii* and *Calophyllum wallichianum* and their Antibacterial Activities" in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the student be awarded the Master of Science.

Members of the Thesis Examination Committee were as follows:

Emilia binti Abd Malek, PhD
Senior Lecturer
Faculty of Science
Universiti Putra Malaysia
(Chairman)

Siti Mariam binti Mohd Nor, PhD
Senior Lecturer
Faculty of Science
Universiti Putra Malaysia
(Internal Examiner)

Chuah Cheng Hock, PhD
Professor
University of Malaya
Malaysia
(External Examiner)

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

Date: 24 May 2018

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

Gwendoline Ee Cheng Lian, PhD
Professor
Faculty of Science
Universiti Putra Malaysia
(Chairman)

Intan Safinar Ismail, PhD
Associate Professor
Faculty of Science
Universiti Putra Malaysia
(Member)

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

Date:

Declaration by graduate student

I hereby confirm that:

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

Signature:_____ Date: _____

Name and Matric No.: Tee Keng Hong (GS47349)

Declaration by Members of Supervisory Committee

This is to confirm that:

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

Signature: _____
Name of Chairman of Supervisory Committee: Professor Gwendoline Ee Cheng Lian

Signature: _____
Name of Member of Supervisory Committee: Assoc. Professor Dr. Intan Safinar Ismail

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

δ	Chemical shift in ppm
%	Percentage
λ_{max}	Wavelength maxima in nm
°C	Degree celcius
^{1}H	Proton
^{13}C	Carbon-13
COSY	Correlation spectroscopy
cm	centimetre
d	doublet
dd	doublet of doublet
DEPT	Distortionless Enhancement by Polarisation Transfer
DMSO	Dimethylsulfoxide
EIMS	Electron Ionisation Mass Spectrometry
FTIR	Fourier Transform Infrared
g	gram
GC	Gas Chromatography
GC-MS	Gas Chromatography-Mass Spectrometry
HMBC	Heteronuclear Multiple Bond Correlation
HMQC	Heteronuclear Multiple Quantum Correlation
Hz	Hertz
IR	Infrared
J	Coupling constant in Hz
kg	kilogram
Lit.	Literature
m	multiplet
M^+	Molecular ion
$M+H^+$	Protonated molecular ion
mg	milligram
µg	microgram
ml	millilitre
mm	millimetre
MHz	megahertz
m.p.	Melting point
MS	Mass spectrum/spectra/spectrometer/spectrometry
m/z	Mass per charge
nm	nanometre
NMR	Nuclear Magnetic Resonance
ppm	parts per million
t	triplet
s	singlet
TLC	Thin Layer Chromatography
TMS	Tetramethylsilane
ν_{max}	Wavenumber maxima in cm^{-1}
UATR	Universal Attenuated Total Reflection
UV	Ultra Violet
UV-Vis	Ultra Violet-Visible

CHAPTER 1

INTRODUCTION

1.1 General Introduction

Plants have been known as remedies provided by nature for treatment of a wide variety of diseases and sicknesses. For instance, the usage of medicinal plants has been recorded by Chinese scholars since three thousand years ago (Cordell, 2009). In our very own country, there are huge areas covered by historical tropical forests. Within these forests, there are about 550 genera of fauna in which 1300 species have been identified to have medicinal values. In fact, the Malaysian market for medicinal plants and herbal products in 1999 is approximately RM4.6 billion (Jamal, 2006). Till this day, there are about 50 medicinal drugs given rise by tropical plants. Newman (2008) estimates that about 60% of the available drugs are derived directly or indirectly from natural products. Surprisingly, the species that have been studied for the pharmaceutical properties to date only make up 1% of the entire entities of plants found in tropical forests (Gurib-Fakim, 2006). Hence, the woods in Malaysia are believed to have a huge potential to provide some of the exotic species that can contribute to the discovery of new medicinal drugs.

Usually, the medicinal properties of plants are due to the presence of secondary metabolites found in the plants. Those compounds can be steroidal alkaloids, saponins, terpenoids, polyphenols and glycosides (Hao *et al.*, 2015). Recently, both chemists and phytochemists have agreed upon the vast diversity of secondary metabolites that are in these plants. In most cases, there are more than 1000 chemical entities contained in each individual plant species (Mitra *et al.*, 2007). The traditional method of utilising these plants as medicines was without the knowledge of these compounds in the plants. However, with the aid of modern scientific knowledge and technology, these compounds can now be studied, identified and mass produced. With the frequent occurrence of disease outbreaks and the existence of antibiotic-resistant pathogens, the urgency of catching up with the development of new drugs is rising. Since healthcare is also one of mankind's necessities to survive, as vital as food, water and shelter, the continuous work to isolate new compounds from plants is important in the development of modern pharmaceutical and medicinal advancement.

1.2 Botany of Plant Studied

1.2.1 The family Clusiaceae

This family contains a total of 30 genera and 1600 species. Plants from the family Clusiaceae are woody evergreen. The leaves are opposite, flowers are actinomorphic and sepals are free or only connate at base. The trees, shrubs or herbs usually produce yellow, orange or clear resinous latex. The different species of several genera are known for producing some common products. For instance, *Garcinia mangostana* (Mangosteen) produces edible fruits, *Callophyllum* provides timber and *Hypericum* provides cultivated ornamentals (Kerrigan *et al*., 2011).

Clusiaceae are known for producing a wide array of isoprenylated xanthones, bioflavonoids and anthraquinones (Stevens, 2007). These phenolic compounds are known to have potent bioactivities. Therefore, Clusiaceae plants are frequently studied from the chemistry aspect. The discovery of potential lead compounds can eventually clear a path for the invention of medicinal drugs for the incurable diseases.
One of the well studied plants is *Calophyllum inophyllum* of genus *Calophyllum*. Despite the extent of studies that have been conducted on this particular plant genus, many novel compounds are still being discovered.

1.2.2 The genus *Calophyllum*

The plants are trees or shrubs mainly distributed in Asia. The latex produced is colourless, white or yellow. The flowers have sepals that are similar to the petals (Steven, 1980). The genus *Calophyllum* is known to contain rich sources of secondary metabolites such as xanthones, coumarins and triterpenes (Patil *et al*., 1993; Kashman *et al*., 1992). Other than that, the plants from this genus also produce some chromanones and steroids (Su, 2008).

The plants have high economic values as well. Trees of *Calophyllum* are a source of timber which can then be made into furniture and decorative items. The fats and oils extracted from the plants are used as medicine as well as in making soap (Stevens, 1980). Besides, some of the species can produce poisonous latex that is used to numb fishes and kill rats (Kawamura *et al.*, 2012). Many plants of this genus are used as traditional herbal medicines. They can be used as a diuretic, for the treatment of malaria, haemorrhoids, chronic ulcers as well as skin infections (Su *et al*., 2008).

1.2.3 The species *Calophyllum andersonii*

There is not much botanical information recorded on this particular plant. This species is mainly distributed in north-western Borneo. The trees can grow up to 30 metres tall with bark that appears reddish or dark brown. The trees also produce latex that is yellow in colour. The twigs are slightly flattened and are 1.3 to 3 mm across. The older twigs are dry striate and pale yellowish brown, in contrast to the blackish petioles. The plant can also be easily recognised by its short, plump terminal buds. This species looks very similar to *Calophyllum teysmanii*. However, one of the noticeable differences is that the filaments of *C. andersonii* appear to be papillated towards the apex (Stevens, 1980). The plant has not been reported in any phytochemical research to date.

Figure 1.1: The tree of *Calophyllum*

1.2.4 The species *Calophyllum wallichianum*

The plant species *Calophyllum wallichianum* is found mainly in peninsular Malaysia, Singapore and Borneo island. The trees can grow up to 36 metres tall. The stem bark is of brown or grey colour, with vertical lines of lenticels and shallowly-fissured. The inner bark is red in colour and the latex produced from the bark is sticky and turns yellowish when exposed. The twigs are 2.5 to 6mm across and are slightly flattened. The flowers consist of four petals, rarely six. The fruits are ellipsoid, oval or spherical. This particular species can be recognised by its terminal over 1cm (Stevens, 1980).

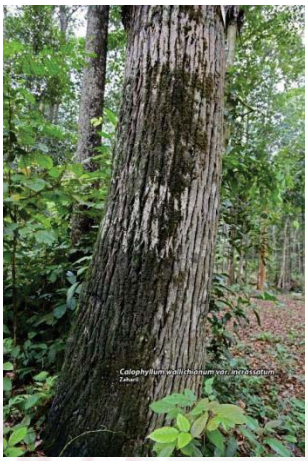

Figure 1.2: Stem bark of *Calophyllum wallichianum*
(Photo courtesy: Mr. Zaharil Dzulkafly)

1.3 Problem Statement

The discovery of new drugs is becoming more important due to the emergence of new diseases and the development of drug resistance by existing harmful pathogens (Cordell, 2002). Many approaches are available nowadays in the pathway of drug discovery. Despite the recent popularity of molecular modelling and other synthetic chemistry techniques, natural products, in particularly medicinal plants, remain as an important source of new drugs, new drugs leads and new chemical entities.

1.4 Objectives of Study

The main objective of the research is to isolate antibacterial compounds which can lead to the discovery of new antibacterial drug candidates for future drug discovery research.

Hence, the specific objectives below are to be achieved in order to fulfil the requirement of the research:

1. To isolate pure compounds from the extracts of *Calophyllum andersonii* and *Calophyllum wallichianum* using column chromatography
2. To elucidate the structures of pure compounds using various spectroscopic methods
3. To investigate the anti-bacterial activities of the plant extracts and pure compounds isolated from the extracts

BIBLIOGRAPHY

Akihisa, T., Yamamoto, K., Tamura, T., Kimura, Y., Iida, T., Nambara, T. & Chang, F. C. (1992). Triterpenoid ketones from *Lingnania chungii* McClure: Arborinone, friedelin and glutinone. *Chemical and Pharmaceutical Bulletin*, 40(3), 789-791.

Alkhamaiseh, S. I., Taher, M., Ahmad, F., Susanti, D. & Ichwan, S. J. A. (2011). Antioxidant and cytotoxic activities of *Calophyllum rubiginosum. International Journal of Phytomedicine*, 3, 157-163.

Amaral, M. C. E., Faria, A. D., Magalhaes, A. F., Magalhaes, E. G. & Ruiz, A. L. T. G. (2004). Steroids and triterpenes from *Eleocharis acutangula* and *E. Sellowiana* (Cyperaceae). *Phytochemical Analysis*, 15, 125-129.

Brenzan, M. A., Nakamura, C. V., Filho, B. P. D., Ueda-Nakamura, T., Young, M. C. M. & Cortez, D. A. G. (2007). Antileishmanial activity of crude extract and coumarin from *Calophyllum brasiliense* leaves against *Leishmania amazonensis. Parasitology research*, 101, 715-722.

Cao, S. –G., Lim, T. –B., Sim, K. –Y. & Goh, S. H. (1997). A highly prenylated xanthone from the bark of *Calophyllum gracilipes* (Guttiferae). *Natural Product Letters*, 10(1), 55-58.

Cao, S. -G., Wu, X. -H., Sim, K. -Y., Tan, B. H. K., Vittal, J. J., Pereira, J. T. & Goh, S. -H. (1998). Minor coumarins from *Calophyllum teysmanii* var. *inophylloide* and synthesis of cytotoxic calanone derivatives. *Helvetica Chimica Acta*, 81, 1404-1416.

Clinical Laboratory Standards Institute (CLSI). 2012. Method for dilution antimicrobial susceptibility test for bacteria that grow aerobically. Approved standard M09-A9. National Committee for Clinical Laboratory Standards, Wayne, PA, USA.

Clinical Laboratory Standards Institute (CLSI). 2012. Performance standard for antimicrobial disc susceptibility. Approved standard M02-A11. National Committee for Clinical Laboratory Standards, Wayne, PA, USA.

Cordell, G. A. (2002). Natural products in drug discovery - Creating a new vision. *Phytochemistry Reviews*, 1(3), 261-273.

Cordell, A. G. (2009). The Convention on Biological Diversity and its impact on Natural Product Research. In *Natural Product Chemistry for Drug Discovery* (pp. 82-138). Illinois, USA: Royal Society of Chemistry.

Daud, S., Ee, G. C. L., Malek, E. A., Teh, S. S. & See, I. (2014). A new coumarin from *Calophyllum hosei. Natural Product Research*, 28(19), 1534-1538.

Dharmaratne, H. R. W., Napagoda, M. T & Tenakoon, S. B. (2009). Xanthones from roots of *Calophyllum thwaitesii* and their bioactivity. *Natural Product Research*, 23(6), 539-545.

Ee, G. C. L., Mah, S. H., Teh, S. S., Rahmani, M., Go, R. & Yap, T. Y. H. (2011). Soulamarin, a new coumarin from stem bark of *Calophyllum soulattri. Molecules*, 16, 9721-9727.

Ee, G. C. L., Ng, K. N., Taufiq-Yap, Y. H., Rahmani, M., Ali, A. M. & Muse, R. (2004). Mucigerin, a new coumarin from *Calophyllum mucigerum* (Guttiferae). *Natural Product Research*, 18(2), 123-128.

Fujita, T., Liu, D. -Y., Ueda, S. & Takeda, Y. (1992). Xanthones from *Polygala tenuifolia. Phytochemistry*, 31(11), 3997-4000.

Guilet, D., Seraphin, D., Rondeau, D., Richomme, P. & Bruneton, J. (2001). Cytotoxic coumarins from *Calophyllum dispar. Phytochemistry*, 58, 571-575.

Gurib-Fakim, A. (2006). Medicinal Plants: Traditions of Yesterday and Drugs of Tomorrow. *Molecular Aspects of Medicine*, 27(1), 1-93.

Ha, L. D., Hansen, P. E., Duus, F., Pham, H. D. & Nguyen, L. -H. D. (2012). A new chromanone acid from the bark of *Calophyllum dryobalanoides. Phytochemistry Letters*, 5, 287-291.

Hao, D. C., Gu, X. J. & Xiao, P. G. (2015). *Medicinal Plants: Chemistry, Biology and Omics*. Cambridge, UK: Woodhead.

Huang, M. X. (2007). Chemistry of *Calophyllum wallichianum, Scapania undulata, Plagiochila colorans* and biotransformation of natural products [master's thesis]. Singapore: National University of Singapore.

Huerta-Reyes, M., Basualdo, M. C., Abe, F., Jimenez-Estrada, M., Soler, C. & Reyes-Chilpa, R. (2004). HIV-1 inhibitory compounds from *Calophyllum brasiliense* leaves. *Biological and Pharmaceutical Bulletin*, 27(9), 1471-1475.

Ida Madiha, Y., Rukayadi, Y., & Norhayati, H. (2017). Effects of extraction conditions on yield, total phenolic contents and antibacterial activity of methanolic *Cinnamomum zeylanicum Blume* leaves extract. *International Food Research Journal*, 24(2), 779-786.

Iinuma, M., Ito, T., Tosa, H., Tanaka, T., Miyake, R., & Chelladurai, V. (1997). New linear pyranoxanthones from *Calophyllum apetalum. Heterocycles*, 45 (2), 299-307.

Iinuma, M., Tosa, H., Tanaka, T.,& Yonemori, S. (1994).Two new xanthones in the underground part of *Calophyllum inophyllum. Heterocycles*, 37 (2), 833-838.

Ito, C., Itoigawa, M., Mishina, Y., Filho, V. C., Enjo, F., Tokuda, H., Nishino, H. & Furukwa, H. (2003). Chemical constituents of *Calophyllum brasiliense*. 2. Structure of three new coumarins and cancer chemopreventive activity of 4-substituted coumarins. *Journal of Natural Products*, 66, 368-371.

Itoigawa, M., Ito, C., Tan, H. T. -W., Kuchide, M., Tokuda, H., Nishino, H. & Furukawa, H. (2001). Cancer chemopreventive agents, 4-phenylcoumarins from *Calophyllum inophyllum. Cancer Letters*, 169, 15-19.

Jamal, J. A. (2006, November). Malay Traditional Medicine: An Overview of Scientific and Technological Process. *Tech Monitor*, 37-49.

Jamaluddin, F., Mohamed, S. & Lajis, M. N. (1994). Hypoglycaemic effect of *Parkia speciosa* seeds due to the synergistic action of β-sitosterol and stigmasterol. *Food Chemistry*, 49(4), 339-345.

Joshi, S. P., Deodhar, V. B. & Phalgune, U. D. (2000). A new coumarin from the seeds of *Calophyllum inophyllum* Linn. *Indian Journal of Chemistry*, 39B, 560-561.

Kashman, Y., Gustafson, K. R., Fuller, R. W., Cardellina, J. H., II, McMahon, J. B., Currens, M. J., Buckheir, R. W., Jr, Hughes, S. H., Cragg, G. M., & Boyd, M. R. (1992). The calanolides, a novel HIV-inhibitory class of coumarin derivatives from the tropical rainforest tree, *Calophyllum lanigerum. Journal of Medicinal Chemistry*, 35, 2735-2743.

Kawamura, F., Muhamud, A., Hashim, R., Sulaiman, O. & Ohara, S. (2012). Two antifungal xanthones from the heartwood of *Calophyllum symingtonianum. Japan Agricultural Research Quarterly*, 46(2), 181-185.

Kawamura, F., Muhamud, A., Hashim, R., Sulaiman, O., & Ohara, S. (2012). Two antifungal xanthones from the heartwood of *Calophyllum symingtonianum. Japan Agricultural Research Quarterly*, 46(2), 181-185.

Kerrigan, R.A., Cowie, I.D. & Dixon, D.J. (2011). Clusiaceae. In Short, P.S. & Cowie, I.D. (eds), *Flora of the Darwin Region*. (Northern Territory Herbarium, Department of Natural Resources, Environment, the Arts and Sport), 1, 1–7.

Khan, M. R., Kihara, M. & Omoloso, A. D. (2002). Antimicrobial activity of *Calophyllum soulattri. Fitoterapia*, 73, 741-743.

Kijjoa, A., Gonzalez, M. J., Pinto, M. M. M., Silva, A. M. S., Anantachoke, C. & Herz, W. (2000). Xanthones from *Calophyllum teysmanii* var. *inophylloide*. *Phytochemistry*, 55, 833-836.

Kuete, V. (2010). Potential of Cameroonian Plants and Derived Products against Microbial Infections: A Review. *Planta Medica*, 76, 1479-1491.

Li, Z. -L., Li, Y., Qin, N. -B., Li, D. -H., Liu, Z. -G. & Liu, Q. (2016). Four new coumarins from the leaves of *Calophyllum inophyllum. Phytochemistry Letters*, 16, 203-206.

Ma, C. -H., Chen, B., Qi, H. -Y., Li, B. -G. & Zhang, G. -L. (2004). Two pyranocoumarins from the seeds of *Calophyllum polyanthum. Journal of Natural Products*, 67, 1598-1600.

Mah, S. H., Ee, G. C. L., Rahmani, M., Yap, T. Y. H., Sukari, M. A. & Teh, S. S. (2011). A new pyranoxanthone from *Calophyllum soulattri. Molecules*, 16, 3999-4004.

Mah, S. H., Ee, G. C. L., Teh, S. S., Rahmani, M., Lim, Y. M. & Go, R. (2012). Phylattrin, a new cytotoxic xanthone from *Calophyllum soulattri. Molecules*, 17, 8303-8311.

McKee, T. C., Fuller, R. W., Covington, C. D., Cardellina, J. H. II., Gulakowski, R. J., Krepps, B. L., McMahon, J. B., & Boyd, M. R. (1996). New pyranocoumarins isolated from *Calophyllum lanigerum* and *Calophyllum teysmanii. Journal of Natural Products*, 59, 754-758.

Ming, M., Zhang, X., Chen, H. -F., Zhu, L. -J., Zeng, D. -Q., Yang, J., Wu, G. -X., Wu, Y. -Z. & Yao, X. -S. (2016). RXRα transcriptional inhibitors from the stems of *Calophyllum membranaceum*. *Fitoterapia*, 108, 66-72.

Mitra, R., Orbell, J. & Muralitharan, M. S. (2007, January). Agriculture - Medicinal Plants of Malaysia. *Asia-Pacific Biotech News*, 11(2), 105-110.

Monache, F. D., Botta, B., Nicoletti, M., Barros Coelho, J. S., & Andrade Lyla, F. D. (1981). Three new xanthones and mecluraxanthone froom *Rheedia benthamiana* Pl. Triana (Guttifereae). *Journal of the Chemical Society, Perkin Transactions 1*, 0, 484-488.

Monache, G. D., Monache, F. D., Waterman, P. G., Crichton, E. G. & Lima, R. A. (1984). Minor xanthones from *Rheedia gardneriana. Phytochemistry*, 23(8), 1757-1759.

Morel, C., Seraphin, D., Teyrouz, A., Larcher, G., Bouchara, J. -P., Litaudon, M., Richomme, P. & Bruneton, J. (2002). New and antifungal xanthones from *Calophyllum caledonicum. Planta Medica*, 68, 41-44.

Nasir, N. M., Rahmani, M., Shaari, K., Kassim, N. K., Go, R., Stanslas, J. & Jeyaraj, E. J. (2013). Xanthones from *Calophyllum gracilipes* and their cytotoxic activity. *Sains Malaysiana*, 42(9), 1261-1266.

Newman, D. J. (2008). Natural products as leads to potential drugs: An old process or the new hope for drug discovery?. *Journal of Medicinal Chemistry*, 51(9), 2589-2599.

Nguyen, L. -H. T., Lai, N. T. D. D. T., Nguyen, L. T. T., Nguyen, H. T., Nguyen, D. M. & Nguyen, L. -H. D. (2016). Thoreliolides A and B, two polyisoprenylated benzoylphloroglucinols derivatives with a new carbon skeleton from the fruits of *Calophyllum thorelii. Tetrahedron Letters*, 57, 2737-2741.

Nguyen, L. -T. T., Nguyen, H. T., Barbic, M., Brunner, G., Heilmann, J., Pham, H. D., Nguyen, D. M. & Nguyen, L. -H. D. (2012). Polyisoprenylated acylphloroglucinols and a polyisoprenylated tetracyclic xanthone from the bark of *Calophylum thorelii. Tetrahedron Letters*, 53, 4487-4493.

Patil, A. D., Freyer, A. J., Eggleston, D. S., Haltiwanger, R. C., Bean, M. F., Taylor, P. B., Caranfa, M. J., Breen, A. L., Bartus, H. R., Johnson, R. K., Hertzberg, R. P., & Westley, J. W. (1993). The inophyllums, novel inhibitors of HIV-1 reverse transcriptase isolated from the Malaysian tree, *Calophyllum inophyllum* Linn. *Journal of Medicinal Chemistry*, 36(26), 4131-4138.

Prasad, J., Shrivastava, A., Khanna, A. K., Bhatia, G., Awasthi, S. K. & Narender, T. (2012). Antidyslipidemic and antioxidant activity of the constituents ioslated from the leaves of *Calophyllum inophyllum. Phytomedicine*, 19, 1245-1249.

Stevens, P. F. (1980, April). A Revision of the Old World Species of *Calophyllum* (Guttiferae). *Journal of the Arnold Arboretum*, 61(2), 117-424.

Su, X. -H., Zhang, M. -L., Li, L. -G., Huo, C. -H., Gu, Y. -C., & Shi, Q. W. (2008). Chemical constituents of the plants of the genus *Calophyllum*. *Chemistry and Biodiversity*, 5, 2579-2608.

Wei, D. -J., Mei, W. -L., Zhong, H. -M., Zeng, Y. -B., Wu, X. D. & Dai, D. F. (2011). A new prenylated xanthone from the branches of *Calophyllum inophyllum. Journal of Asian Natural Products Research*, 13(3), 265-269.

Wolform, M. L., Dickey, E. E., McWain, P., Thompson, A., Looker, J. H., Windrath, O. M. & Komitsky, F. Jr,. (1964). Osage orange pigments. XIII. Isolation of three new pigments from the root bark. *The Journal of Organic Chemistry*, 29(3), 689-691.

Xiao, Q., Zeng, Y. -B., Mei, W. -L., Zhao, Y. -X., Deng, Y. -Y. & Dai, H. F. (2008). Cytotoxic prenylated xanthones from *Calophyllum inophyllum. Journal of Asian Natural Products Research*, 10(10), 993-997.

Yimdjo, M. C., Azebaze, A. G., Nkengfack, A. E., Meyer, A. M., Bodo, B. & Fomum, Z. T. (2004). Antimicrobial and cytotoxic agents from *Calophyllum inophyllum. Phytochemistry*, 65, 2789-2795.

Zou, J., Wu, J., Liu, S. -Z. & Zhao, W. -M. (2010). New coumarins and triterpenes from *Calophyllum inophyllum. Helvetica Chimica Acta*, 93, 1812-1821.