UNIVERSITI PUTRA MALAYSIA

CHEMICAL CONSTITUENTS AND BIOLOGICAL ACTIVITIES OF CALOPHYLLUM VENULOSUM ZOLL. AND CALOPHYLLUM INOPHYLLUM L.

AHMAD AZRI FITRI BIN ISMAIL

FS 2016 59

CHEMICAL CONSTITUENTS AND BIOLOGICAL ACTIVITIES OF *CALOPHYLLUM VENULOSUM* ZOLL. AND *CALOPHYLLUM INOPHYLLUM* L.

By

AHMAD AZRI FITRI BIN ISMAIL

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

August 2016

COPYRIGHT

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

CHEMICAL CONSTITUENTS AND BIOLOGICAL ACTIVITIES OF *CALOPHYLLUM VENULOSUM* ZOLL. AND *CALOPHYLLUM INOPHYLLUM* L.

By

AHMAD AZRI FITRI BIN ISMAIL

August 2016

Chairman : Prof. Gwendoline Ee Cheng Lian, PhD
Faculty : Science

A phytochemical investigation on the stem bark of two species from the Clusicaceae family, Calophyllum venulosum and Calophyllum inophyllum was carried out using various chromatographic techniques and xanthones, coumarin, terpenes and sterol were successfully isolated and identified. All of the secondary metabolites isolated were structurally characterized on the basis of modern spectroscopic evidence, such as 1D and 2D NMR, MS, IR and UV.

Extensive chromatographic separations applied to the chloroform extract of the stem bark of Calophyllum venulosum resulted in one new xanthone; venuloxanthone, along with two other xanthones, caloxanthone I and tovopyrifolin C. The hexane extract afforded two pentacyclicterpenes; friedelin and lupeol.

Meanwhile, purifications of the chloroform extract revealed the presence of a coumarin, benjaminin together with a xanthone, macluraxanthone. Two sterols, stigmasterol and β-sitosterol were also discovered to be present in the hexane extract.

The crude extracts of both plants were screened for cytotoxic and antimicrobial activities. The cell lines used in the cytotoxic assays were human promyelocytic leukemia (HL60) and positive breast cancer (MCF7). For *Calophyllum venulosum*, only the methanol extract showed significant activity against MCF-7, meanwhile the hexane and chloroform extracts from *Calophyllum inophyllum* were found to be active against both cell lines. The antimicrobial activities was carried out on *Staphylococcus aureus* S276, *Bacillus subtilis* B145,

Staphylococcus epidermidis S273, *Escherichia coli* E266 and *Serratia marcencens* S381. From the tests, only the hexane and chloroform crude extracts from *Calophyllum inophyllum* showed weak inhibition against *Bacillus subtilis* B145, while no activity were indicated by the other crude extracts.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Sarjana Sains

SEBATIAN KIMIA DAN AKTIVITI BIOLOGI DARIPADA KULIT *CALOPHYLLUM VENULOSUM* ZOLL. DAN *CALOPHYLLUM INOPHYLLUM* L.

Oleh

AHMAD AZRI FITRI BIN ISMAIL

Ogos 2016

Pengerusi : Prof. Gwendoline Ee Cheng Lian, PhD
Fakulti : Sains

Satu kajian fitokima terhadap kulit dua spesies daripada keluarga Clusicaceae, iaitu Calophyllum venulosum dan Calophyllum inophyllum telah dijalankan menggunakan pelbagai teknik kromatografi, dan berjaya menemui xanton, kumarin, terpene, dan sterol. Struktur kesemua metabolit sekunder yang diasingkan dikenalpasti dengan analisi spektroskopi moden seperti 1D dan 2D, NMR, MS, IR and UV.

Teknik kromatografi yang dijalankan keatas ekstrak klorofom daripada kulit batang Calophyllum venulosum memberikan satu xanton baru, diberi nama venuloxanthone, disamping dua xanton lain iaitu caloxanthone I dan tovopyrifolin C, manakala ekstrak hexane memberi dua terpene iaitu fridelin dan lupeol.

Sementara itu, kajian keatas kulit batang Calophyllum inophyllum menunjukkan kehadiran koumarin dan satu xanton, iaitu macluraxanthone dalam ekstrak klorofom. Dua sterol, iaitu stigmasterol dan β-sitosterol dijumpai di dalam ekstrak heksana.

Ekstrak dari kedua-dua pokok diuji dengan aktiviti sitotoksik dan antimikrob. Sel-sel yang digunakan dalam aktiviti sitotoksik ialah sel leukemia promeilositik manusia (HL60) dan kanser payudara reseptor positif estrogen manusia (MCF7). Hanya metanol ekstrak daripada Calophyllum venulosum menunjukkan adanya aktiviti menentang MCF-7, manakala untuk Calophyllum inophyllum, heksana dan klorofom ekstrak didapati aktif menentang kedua-dua sel. Aktiviti antimikrob diuji dengan Staphylococcus aureus S276, Bacillus subtilis B145, Staphylococcus epidermidis S273, Escherichia coli E266 dan Serratia marcencens S381. Hasilnya, hanya heksana dan kloroform ekstrak daripada

Calophyllum inophyllum menunjukkan sedikit aktiviti menentang Bacillus subtilis B145, manakala lain-lain ekstrak tidak menunjukkan sebarang aktiviti.

ACKNOWLEDGEMENTS

BISMILLAHIRAHMANIRRAHIM….

First and foremost, I would like to thank my parents, Ismail bin Daud and Noriah bte Abd Rani for their endless love, prayers and for continuously supporting me emotionally throughout my study. Thank you both for giving me strength to reach for the stars and chase my dreams. My sisters and little brothers deserve my wholehearted thanks as well.

I also would like to express my greatest gratitude to my supervisor, Prof. Dr. Gwendoline Ee Cheng Lian for giving me a chance to do my research under her guidance. She has helped me a lot, to make sure that I can finish my research. With her encouragement, guidance, advise, patience and support throughout my study in Universiti Putra Malaysia, I finally complete my reserach. I also would like to thank my co-supervisor, Prof. Dr. Mohd Aspollah Hj. Sukari for providing me with a lot of help. I sincerely thank all staff in the Chemistry Department, Faculty of Science, especially Mr. Johadi and Mrs. Shareena for NMR analysis, Mr. Zainal for recording the MS and Mrs. Rusnani for IR analysis.

My thanks goes to my labmates, Syarik, Abang Shaari, Irene, Ain and also Dr. Teh for their help in this research. Not forgetting my friend, Nazil for his help in sending my samples to Dr. Najihah for bioassay tests, and also a really special thanks for my best friend, Azeeza who always stand by me and encouraging me when I lost hope, willing to listen and share my problems over the past 4 years.

Last, but not least, I would like to thank someone special, Nur Hazera bte Md Radzi for her understanding and endless love during the past few months. Her support and encouragement was in the end what made this thesis possible.

May Allah bless all of us.

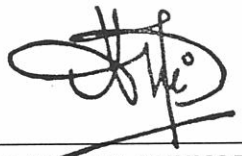
I certify that a Thesis Examination Committee has met on 24 August 2016 to conduct the final examination of Ahmad Azri Fitri bin Ismail on his thesis entitled "Chemical Constituents and Biological Activities of *Calophyllum venulosum* Zoll. and *Calophyllum inophyllum* L." in accordance with the Universities 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:

Tan Yen Ping, PhD
Senior Lecturer
Faculty of Science
Universiti Putra Malaysia
(Chairman)

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

Khalijah Awang, 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: 3 November 2016

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)

Mohd Aspollah b Sukari, PhD
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 original work;
- quotations, illustrations and citations have been duly referenced;
- this thesis has not been submitted previously or concurrently for any other degree at any 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 (Research and Innovation) before thesis is published (in the form of written, printed or in electronic form) including books, journals, modules, proceedings, popular writings, seminar papers, manuscripts, posters, reports, lecture notes, learning modules or any other materials as stated in the Universiti Putra Malaysia (Research) Rules 2012;
- there is no plagiarism or data falsification/fabrication in the thesis, and scholarly integrity is upheld as according to the Universiti Putra Malaysia (Graduate Studies) Rules 2003 (Revision 2012-2013) and the Universiti Putra Malaysia (Research) Rules 2012. The thesis has undergone plagiarism detection software.

Signature: _____ Date: _____

Name and Matric No.: _____

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: Gwendoline Ee
Name of Chairman of Supervisory Committee: PROF. DR. Gwendoline Ee Cheng Lian

Signature: Gwendoline Ee
Name of Member of Supervisory Committee: b/p Dr. Mohd Aspollah Hj Md Sukari

Signature: _____
Name of Member of Supervisory Committee: _____

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER 1

INTRODUCTION

1.1 General Introduction

Plants which are gifts from God have been the source of many cures for illnesses for over the centuries. Plants also offer essential materials for shelter, furniture, food, clothing, writing and colouring materials and weapons (Balick et al., 1996). In the early years, traditional medical practitioners sold medicinal plants in the crude form based on the knowledge they gain from the old folks. In the 1950's, scientific research were established. Scientists started to carry out research on medicinal plants seriously. The first report on a phytochemical survey of plants in Malaysia was carried out by Arthur in 1954 which led to more investigations until today (Teo et al., 1990).

Malaysia has been known for its richness in flora and fauna. Its position near the equator which gives it a typical tropical climate, characterized by high temperatures, humidity and rainfall throughout the year has resulted in a rich and beautiful flora and fauna. It was estimated that Malaysia is home to some 15 000 species or higher plants. Malaysia is listed as the 12th most diverse nation in the world and is mainly covered by tropical rainforests. About 25% of all modern drugs originate from rainforests even though only less than 1% of the world's tropical rainforest plant species have been evaluated for pharmacological properties. Malaysian forest is a rich source of numerous classes of natural compounds such as alkaloids, anthraquinones and phenolic compounds.

The phytochemical study of several well-known plants in folklore medicine such as *Eurycoma longifolia* Jack (Tongkat Ali), *Labisa pumila* (Kacip Fatimah), and *Morinda citrifolia* (noni) yielded many bioactive phytochemicals with good biological activities. A study in 2000 gave evidence of the folk use of *E.longifolia* as an aphrodisiac (Ang et al., 2000). On the other hand, *Labisa pumila* is effective as a skin collagen synthesis promoting herb (Chua et al., 2012) while *Morinda citrifolia* shows antibacterial activity (Atkinson, 1956).

Natural product chemistry is referred to as the study on secondary metabolites produced by living organisms such as plants. Due to their medicinal properties, natural compounds are usually preferred as alternative drugs rather than synthetic drugs used in pharmaceutical industry nowadays. The ancient cultures are known for their systematic collection of information on herbs and their rich and well-defined herbal pharmacopoeias (Mohagheghzadeh et al., 2006). Before synthetic drugs were found, most medicines were obtained from leaves, roots, bark and seeds of plants. The World Health Organization reported that 80% of the population in developing countries depend on traditional medicine for their primary health care. The dominant role played by medicinal plants contributed approximately 60% of anti-cancer compounds and 75% of drugs for infectious

diseases, either from natural products or natural product derivatives (Newman et al., 2003, Shoeb et al., 2006).

This research was conducted due to the advancement in the field of natural products. *Calophyllum venulosum* and *Calophyllum inophyllum* were chosen in this research in our search for bioactive compounds. Previous studies on *Calophyllum* species have shown anti-microbial (Yimdjo et al., 2004), anti-oxidant (Dharmaratne et al, 2009) and anti-cancer (Itoigawa et al, 2001).There should be at least two subheadings to justify having subheadings.

1.2 Botany of Plants Studied

1.2.1 The Family Clusicaceae

Clusiaceae, previously named as the Guttiferae which means "latex bearing plant" grows widely in the Malaysian forests. *Garcinia*, *Calophyllum*, and *Mesua* are some of the genera discovered under this family. This family of plants usually gives a number of useful timbers, drugs, dyes, gums pigments and resins. The Clusiaceae family consists of about 40 genera and over 1600 species of trees and shrubs and are easily found in Tropical Asia and Africa. In Malaysia, the genera that are mainly found are *Calophyllum*, *Mesua*, *Mammea*, and *Garcinia*.

Members of the Clusiaceae family can be recognised as trees, shrubs, or sometimes herbs containing resin or oil in canals and sometimes black or red glands containing hypericin or pseudohypericin. They also have sticky sap and fruits or capsules used for seeds and flowers are bisexual or unisexual with separate sepals and petals. The flowers also appear in bright colours while the leaves are always simple, oblong in shape with horizontal veins.

1.2.2 The Genus *Calophyllum*

The name *Calophyllum* originated from the Greek language where "kalos" means beautiful and "phullon" means leaf. This genus is composed of a great group of tropical tress. There are approximately 180-200 species restricted to the hot and humid tropics and can be found in Australasia, Madagascar, Eastern Africa, South and Southeast Asia, the Pacific islands, the West Indies and Latin America (Morel et al., 2000). Many of these species are employed in popular medicines to treat injuries such as pain, infection, inflammation and ulcer. (Dharmaratne et al., 1999). The genus *Calophyllum* has around 180-200 species of tropical evergreen trees and are widely distributed in Australasia, Madagascar, Eastern Africa, South and Southeast Asia, the Pacific islands, the West Indies and Latin America (Morel et al., 2000).

Calophyllum is also known as "bintangor" locally. (Corner 1998; Whitmore 1973). It usually has a hard and rough bark while its leaves are shiny and leathery. It can reach up from 30 to 60 m in height. On the other hand, it also has fragrant

flowers to attract insects or birds for pollinating purposes. The flowers also are the main source of seed oil production (Dweck et al., 2002).

Figure 1.1 : *Calophyllum* tree

Figures 1.2 : Stem and bark of *Calophyllum* species

Figures 1.3 : Fruits of *Calophyllum* species

Figures 1.4 : Flowers of *Calophyllum* species

1.2.3 The Species *Calophyllum venulosum*

Calophyllum venulosum is a dicotyledenous plant species discovered by Heinrich Zollinger. This plant can be found in Java, Sumatera, Philippine and Malaysia. However, there is not much research carried out on this species. So far, there are only two investigations on this species (Cao et al., 1997, Cao et al., 2001) which resulted in the isolation of new biflavanoids but there is no report on its biological activities. Thus, this plant has been chosen to discover more new bioactive compounds.

1.2.4 The Species *Calophyllum inophyllum*

Calophyllum inophyllum, is also known as *kamani* in Hawaii and *tamanu* in Tahiti. This plant is usually found in warm tropical and sub-tropical areas. It can reach a height of about 8-30 m and its fragrant flower is 25 mm wide. The diameter of the fruit is about 2 to 5 cm and it is round shaped, while its colour varies from yellow to brownish-red. This plant is usually used for medicinal and ornamental purposes due to its fragrant white flowers and glossy leaves, and provides shelter on streets and along the beaches.

1.3 Problem Statement

Natural products screening from *Calophyllum* species can lead to the discovery of new potential anticancer leads that are of great importance to pharmaceutical industry and public health which are not accessible by other technologies. The bioassay results and the knowledge in bioassay screening, as well as isolation techniques and structural elucidation of new compounds will be useful information for future advanced study by natural products scientists in industries worldwide. The discovery and usage of these possible potential anticancer agents can serve as alternatives to the presently available drugs in the market.

1.4 Objectives of Study

This research was designed to carry out extraction, isolation, characterization, elucidation and evaluation of the secondary metabolites present in the extracts of *Calophyllum venulosum* and *Calophyllum inophyllum*. The discovery of new bioactive compounds and their structures is the ultimate goal for this research.

As such, the following specific objectives are to be met as the goals of the project:

1. To extract and isolate chemical constituents from the stem bark of *Calophyllum venulosum* and *Calophyllum inophyllum.*

2. To characterize and elucidate the structures of the isolated compounds using various modern spectroscopic techniques, such as NMR, MS and IR.

3. To evaluate the biological activities of the crude extracts using bioassays; cytotoxic and antimicrobial.

BIBLIOGRAPHY

Ali, A.M., Mackeen, M.M., Safinar, I., Hamid, I., Lajis, N.H. (1996). Antitumor promoting and antitumor activities of the crude extract from the leaves of *Juniperus chinensis*. *Journal of Ethnopharmacology*. 53: 165-169.

Ang, H.H., Cheang, S.H., Yusof, A.P.M. (2000). Effects of *Eurycoma longifolia* Jack (Tongkat Ali) on the initiation of sexual performance of inexperienced castrated male rats. *Exp. Anim*. 49 (1): 35-38.

Atkinson, N. (1956). Antibacterial substances from flowering plants. *Australian Journal of Experimental Biology & Medical Science*. 34 (1): 17-26.

Balick M.J, Cox P.A.R. (1996). Plants people and culture. The science of Ethnobotany. *Scientific American Library, New York, USA*. 219.

Bennett, G.J., Lee, H.H. (1989). Xanthones from Guttiferae. *Phytochemistry*. 28: 967–998.

Cao, S.G., Sim, K.Y., Goh, S.H. (1997). Biflavanoids of *Calophyllum venulosum*. *Journal of Natural Products*. 60: 1245-1250.

Cao, S.G., Sim, K.Y., Goh, S.H. (2001). Minor methylated pyranoamentoflavones from *Calophyllum venulosum*. *Natural Product Letters*. 15 (5): 291-297.

Cheng, H.C., Wang, L.T., Khalil, A.T., Chang, Y.T., Lin, Y.C., Shen, Y.C. (2004). Pyranoxanthones from *Calophyllum inophyllum*. *Journal of Chinese Chemical Society*. 5: 431-435.

Chilpa, R.R., Muniz, E.E., Apan, T.R., Amekraz, B., Aumelas, A., Jankowski, C.K., Torres, M.V. (2004). Cytotoxic effects of mammea type coumarins form *Calophyllum brasiliense*. *Life Sciences*. 75 (2004): 1635-1647.

Chua, L.S., Lee, S.Y., Abdullah, N., Sarmidi, M.R. (2012). Review on *Labisia pumila* (Kacip Fatimah): Bioactive phytochemicals and skin collagen synthesis promoting herb. *Fitoterapia*. 83 (8): 1322-1335. Corner, E.J.H. (1998). Wayside trees of Malaya. *The Malayan Nature Society, Malaysia*.

Crombie, L., Games, D.E. (1966). Isolation and structure of mammea A/BA, A/AB and A/BB: A group of 4-aryl-coumarin extractives of *Mammea americana* L. *Tetrahedron Letters*. 7 (2): 145–149.

Crombie, L., Games, D.E. (1967). Extractives of *Mammea americana* L Part II. The 4-phenylcoumarins. Isolation and structure of mammea A/AA, A/A cyclo D, A/BA, A/AB and A/BB. *Journal of the Chemical Society*. 7 (C): 2553–2559.

Crombie, L., Games, D.E., Haskins, N.J., Reed, G.F. (1972). Extractives of *Mammea americana* L Part V. The insecticidal compounds. *Journal of the Chemical Society Perkin Transactions I*: 2255–2260.

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

Dharmaratne, H.R.W., Sajeevani, J.R.D.M., Marasinghe, G.P.K., Ekanayake, E.M.H.G.S. (1997b). Distribution of pyranocoumarins in *Calophyllum cordatooblongum*. *Phytochemistry*. 49 (4): 995-998.

Dharmaratne, H.R.W., Sotheeswaran, S., Balasubramaniam, S., Reisch, J. (1985). Xanthones from roots of three *Calophyllum* species. *Phytochemistry*. 25 (8): 1957-1959.

Dharmaratne, H.R.W., Sotheeswaran, S., Balasubramaniam, S., Waight, E.S. (1984). Triterpenoids and coumarins from the leaves of *Calophyllum cordatooblongum*. *Phytochemistry*. 24 (7): 1553-1556.

Dharmaratne, H.R.W., Wijesinghe, W.M.N. (1997a). A trioxygenated diprenylated chromenxanthone from *Calophyllum moonii*. *Phytochemistry*. 46 (7): 1293-1295.

Dharmaratne, H.R.W., Wijesinghe, W.M.N.M., Thevanasem, V. (1999). Antimicrobial activity of xanthones from *Calophyllum* species, against methicillin-resistant *Staphylococcus aureus* (MRSA). *Journal of Ethnopharmacology*. 66 (1999): 339-342.

Dweck, A.C., Meadows, T. (2002). Tamanu (*Calophyllum inophyllum*) – the African, Asian, Polynesian and Pacific Panacea. *Int J Cosmet Sci*. 24 (6): 341-348.

Ee, G.C., Kua, A.S., Lim, C.K., Jong, V. and Lee, H.L. (2006). Inophyllin A, a new pyranoxanthone from *Calophyllum inophyllum* (Guttiferae). *Natural Product Research*. 20(5): 485-491.

Ee, G.C.L., Kua, A.S.M., Cheow, Y.L., Lim, C.K., Jong, V. and Rahmani, M. (2004a). A new pyranoxanthone inophyllin B from *Calophyllum inophyllum*. *Natural Product Sciences*. 10(5): 220-222.

Ee, G.C.L., Mah, S.H., Rahmani, M., Taufiq-Yap, Y.H., Teh, S.S. and Lim, Y.M. (2011a). A new furanoxanthone from the stem bark of *Calophyllum inophyllum*. *Journal of Asian Natural Products Research*. 13(10): 956 960.

Ee, G.C.L., Mah, S.H., Teh, S.S., Rahmani, M., Go, R. and Taufiq-Yap, Y.H. (2011b). Soulamarin, a new coumarin from stem bark of *Calophyllum soulattri*. *Molecules*. 16(11): 9721-9727.

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

Fuller, R.W., Bokesch, H.R., Gustafson, K.R., McKee, T.C., Cardellina II, J.H., McMahon, J.B., Cragg, G.M., Soejarto, D.D., Boyd, M.R. (1994). HIV inhibitory coumarins from latex of tropical rainforest tree *Calophyllum teysmannii* var inophylloide. *Bioorg Med. Chem. Lett*. 4: 1961–1964.

Goh, S.H., Jantan, I., Waterman, P.G. (1992). Neoflavonoid and biflavonoid constituents of *Calophyllum inophyllum*. *Journal Natural Product*. 55: 1414–1420.

Govindachari, T.R., Prakash, D., Viswanthan, N. (1967). Chemical constituents of *Calophyllum apetalum* Willd. *Tetrahedron*. 42: 4177-4181.

Govindachari, T.R., Viswanthan, N., Pai, B.R., Ramadas Rao, U., Srinivasan, M. (1967). Triterpenes of *Calophyllum inophyllum* Linn. *Tetrahedron*. 23: 1901-1910.

Gustafaon, K.R., Bokesch, H.R., Fuller, R.W., Cardellina II, J.H., Kadashin, M.R., Soejarto, D.D., Boyd, M.R. (1994). Calanone, a novel coumarin from *Calophyllum teysmannii*. *Tetrahedron*. 35 (32): 5821-5824.

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 (2012): 287-291.

Hill, R.A., Kirk, D.N., Makin, H.L.J., Murphy, G.M. 1991. Dictionary of Steroids. *Great Britain: Chapman & Hall*.

Iinuma, M., Ito, T., Tosa, H., Tanaka, T., Miyake, R., Chelladurai, V. (1997). Prenylated xanthonoids from *Calophyllum apetalum*. *Phytochemistry*. 46 (8): 1423-1429.

Iinuma, M., Tosa, H., Tanaka, T., Yonemori, S. (1994). Two xanthones from root bark of *Calophyllum inophyllum*. *Phytochemistry*. 35 (2): 527-532.

Iinuma, M., Tosa, H., Toriyama, N., Tanaka, T., Ito, T., Chelladurai, V. (1996). Six xanthones from *Calophyllum austroindicum*. *Phytochemistry*. 43 (3): 681-685.

Ishikawa, T., Oku, Y., Tanaka, T., Kumamoto, T. (1999). An approach to anti HIV active *Calophyllum* coumarin synthesis: An enantioselective construction of 2,3-dimethyl-4-chromanone ring by quinine-assisted intramolecular Michael-type addition. *Tetrahedron*. 40 (1999): 3773 3780.

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 Letter*. 169 (1): 15–19.

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

Karunanayake, S., Sotheeswaran, S., Sultanbawa, M.U.S. (1979). Zeyloxanthone, a new tetrahydroxanthone from *Calophyllum zeylanicum* (Guttiferae). *Tetrahedron*. 16: 1449-1450.

Kawazu, K., Chigashi, H., Mitsui, T. (1968). The piscicidal constituents of *Calophyllum inophyllum* Linn. *Tetrahedron*. 19: 2383-2385.

Kawazu, K., Hajime, O., Norio, T., Tetsuo, M. (1972). Piscicidal constituents of *Calophyllum Inophyllum*. *Bulletin of the Institute for Chemical Research, Kyoto University*. 50 (3): 160-167.

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

Kijjoa, A., Gonzalez, M.J., Pinto, M.M., Silva, A.M., Anantachoke, C., Herz, W. (2000). Xanthones from *Calophyllum teysmannii* var. inophylloide. *Phytochemistry*. 55(7): 833-836.

Li, Y.Z., Li, Z.L., Yin, S.L., Shi, G., Liu, M.S., Jing, Y.K., Hua, H.M. (2010). Triterpenoids from *Calophyllum inophyllum* and their growth inhibitory effects on human leukemia HL-60 cells. *Fitoterapia*. 81 (2010): 586 589.

Mah, S.H., Ee, G.C.L., Rahmani, M., Taufiq-Yap, Y.H., Sukari, M.A. and Teh, S.S. (2011). A new pyranoxanthone from *Calophyllum soulattri*. *Molecules*. 16 (5): 3999-4004.

Mesquita, A.A.L., Oliveira, W.G.D., Neiva, R.M.T., Gotilieb, O.R. (1974). Xanthones from *Tovomita pyrifolium*. *Phytochemistry*. 14: 803-806.

Mohagheghzadeh, A., Faridi, P., Ardakani, M.S., Ghasemi, Y. (2006). Medicinal smokes. *Journal of Ethnopharmacology*. 108 (2): 161-184.

Morel, C., Seraphin, D., Oger, J.M., Litaudon, M., Sevenet, T., Richomme, P. and Bruneton, J. (2000). New xanthones from *Calophyllum caledonicum*. *Journal of Natural Products*. 63 (11): 1471-1474.

Mosmann, T. (1983). Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. *Journal of Immunological Methods*. 65: 55-63.

Newman, DJ., Cragg, G.M., Snader, KM. (2003). Natural products as sources of new drugs over the period 1981–2002. *Journal of Natural Product*. 66: 1022-1037.

Nigam, S.K., Banerji, R., Rebuffat, S., Cesario, M., Pascard, C., Bodo, B. (1988). Soulattrone A, a C_{24} terpenoid from *Calophyllum soulattri*. *Phytochemistry*. 27 (2): 527-530.

Nigam, S.K., Mitra, C.R. (1967). Constituents of *Calophyllum tomentosum* and *Calophyllum apetalum* nuts : structure of a new 4-alkyl and of two new 4-phenylcoumarins. *Tetrahedron*. 28: 2633-2636.

Peres, V., Nagem, T.J. (1997). Trioxygenated naturally occurring xanthones. *Phytochemistry*. 44 (2): 191-214.

Ravelonjato, B., Kunesch, N., Poisson, J.E. (1987). Neoflavanoids from the stem bark of *Calophyllum verticillatum*. *Phytochemistry*. 26 (11): 2973 2976.

Sahimi, M.S.M., Ee, G.C.L., Mahaiyddin, A.G., Daud, S., The, S,S., See, I., Sukari, M.A. (2015). A New Natural Product Compound Benjaminin from *Calophyllum benjaminum*. *Pertanika Journal Tropical Agricultural Science*. 38 (1): 1-6.

Shoeb, M. (2006). Anticancer agents from medicinal plants. *Bangladesh Journal of Pharmacology*. 1: 35-41.

Somanathan, R., Sultanbawa, S., Uvais, M. (1974). Chemical investigation of Ceylonese plants. Part VIII. Trapezifolixanthone, a new di isoprenylated xanthone from the bark of *Calophyllum trapezifolium* Thw. (Guttiferae). *J. Chem. Soc. Perkin Trans*. *I*. 2515–2517.

Taher, M., Idris, M.S., Ahmad, F., Arbain, D. (2005). A polyisoprenylated ketone from *Calophyllum enervosum*. *Phytochemistry*. 66 (2005): 723 726.

Teo, L.E., Pachiaper, G., Chan, K.C., Hadi, H.A., Weber, J.F., Deverre, J.R., David, B., Sevenet, T. (1990). A new phytochemical survey of Malaysia V. Preliminary screening and plant chemical studies. *J. Ethnopharmacol*. 28 (1): 63-101.

Whitmore, T.C. (1973). Tree flora of Malaya vol 2. *Malayan Forest Records*.

Yimdjo, M.C., Azabaze, A.G., Nkengfack, A.E., Meyer, A.M., Bodo, B., Fomum, Z.T. (2004). Antimicrobial and cytotoxic agents from *Calophyllum inophyllum*. *Phytochemistry*. 65 (20): 2789-2795.